F I B R I N O L Y S I S

and its relationship to

AGE

and to

CARCINOMA OF THE PROSTATE

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by

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C O N T E N T S

PREFACE

The work presented here has been performed by me between 1956 and 1961 while holding the appointment of Assistant Haematologist to the United Sheffield Hospitals, and has been carried out in addition to routine clinical and laboratory duties. The patients whose specimens of blood have been examined were attending the Royal Infirmary and the Royal Hospital (including Fulwood Annexe) Sheffield. I am greatly indebted to all the surgeons and physicians who allowed me clinical access to their patients. The majority of these patients were under the care of Mr. J. C. Anderson, Mr. G. W. Blomfield, Professor R. P. Jepson and Professor A. W. Kay. Many other members of the medical and nursing staffs of these hospitals have been equally helpful.

All my laboratory work has been carried out in the Haematology Department of the Royal Infirmary, Sheffield, which is in the charge of Dr. E. K. Blackburn, whose assistance has always been greatly appreciated and without whose co-operation the work reported here would have been impossible. All the fibrinolysis assays in the latter years when the main part of the work was done, and most of the early assays, were performed without assistance, either in the preparation of the equipment or in the performance of the tests. Some technical assistance in the experimental stages of the assays of fibrinolysis was

received from Miss Pauline Hall and from the late Mr. A. Howard This is gratefully acknowledged.

I am indebted to Dr. A. Jordan, Chemical Pathologist to the United Sheffield Hospitals, for most of the plasma fibrinogen estimations and for the serum phosphatase estimations. Mr. D. A. Podmore, of the same department, has taken a particular interest in the plasma fibrinogen estimations.

I should like also to thank Professor D. H. Collins and Dr. J. L. Edwards for such reports as have been quoted concerning histological evidence and autopsy examinations.

Assistance with the assessment of statistics has been acknowledged in appropriate sections of the text. Acknowledgment is particularly due to Dr. G. H. Jowett, now of Melbourne, Australia, to Mr. D. Kerridge, lecturer in the Department of Statistics, University of Sheffield, and also to Mr. M. C. Cliffe for assistance with the calculations.

I should like to thank the Board of Governors of the United Sheffield Hospitals for a grant from the Endowment Fund from which was purchased the major equipment necessary for the work.

In the preparation of this thesis I should like to acknowledge the assistance of Mr. A. S. Foster, who has made my figures suitable for photography, and Mr. J. F. V. Larway for the photography, and finally Miss Sheila Fletcher for the typing.

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DEFINITION OF FIBRINOLYSIS

"Fibrinolysis" is a term used here to describe the process of aseptic dissolution of fibrin or whole blood clot, by means of a lytic agent or of lytic agents contained in the blood.

INTRODUCTION

The value of the clotting of blood has been assumed for centuries and has been generally accepted. Yet if clotting were uncontrolled, its mechanism would be of no value, indeed it would threaten life. It is not surprising, therefore, that there has been shown to exist, amongst other controlling mechanisms, a system of clot dissolution which can counteract the effects of clotting. Its presence, both in health and in disease, has been demonstrated many times but its true significance is still a matter for conjecture.

Aseptic clot dissolution, or fibrinolysis, has been recognised for well over a hundred years, but it was not until Macfarlane and Biggs' review of the subject in 1948 that attention was focussed on the theoretical problem involved, together with its possible clinical importance. At first fibrinolysis could be demonstrated only in subjects under stress. When techniques improved, it could be shown *in vitro* that fibrinolysis occurred in healthy people, but this was not proof of real activity in the body. Finally the hypothesis was put forward (Copley, 1957) that fibrinolysis is not so much a system to be called upon as the need arises, as one which is constantly at work maintaining equilibrium with the so-called endo-endothelial fibrin film which is believed to be the physiological lining of all blood vessels.

Excessive bleeding has been attributed to over-activity of the fibrinolytic system. Nilsson, Sjoerdsma and Waldenström (1960) wrote that they had seen in recent years 43 cases with severe fibrinolysis as the cause of "haemorrhagic symptoms". Here, therefore, is a field where more knowledge of the mechanism of fibrinolysis might lead to proof of a causal relationship and perhaps to better diagnosis and treatment.

Underactivity of the fibrinolytic system appears to be well demonstrated by hyaline membrane disease of the lung. This is usually congenital in origin but may be acquired, and is discussed independently. With less positive evidence, it is considered that underactivity or failure in the fibrinolytic system may well play a part in intravascular thrombosis. Although unproved, this has a very great clinical implication. When intravascular thrombosis takes place, premature clotting is occurring, but it is possible that the clot is allowed to develop because of a fundamental failure of the normal action of the fibrinolytic system. This might have been expected to remove the early fibrin strands, so halting the development of the thrombus. Some evidence has been published to support this theory (Smith and Yates, 1955: Hume, 1958: Nestel, 1959: Czarniecki, 1960: Merskey, Gordon and Lackner, 1960: McDonald, 1960: Tsitouris, Sandberg, deLeon, Lecks and Bellet, 1960).

The fibrinolytic system has therefore been incriminated in dangerous bleeding due to an <u>excess</u> of activity; and in

intravascular clotting and other disorders due to an insufficiency of activity.

In addition, there is almost certainly a place in clinical practice for the administration of fibrinolytic agents as therapeutic substances, to boost or to supersede inadequate fibrinolysis in dealing with an overwhelming local deposition of fibrin. The potential role of fibrinolysis is therefore great, and covers both preventive and therapeutic medicine.

Much work has been done on the problem of fibrinolysis, particularly in the last ten years, but no great advance in practical management appears yet to have arisen and many fundamental points have remained unexplained. Here a short review of published work on fibrinolysis will be given as a background to the presentation of new work. The incidence of active fibrinolysis in carcinoma of the prostate will then be compared to that of a large control series, and an explanation offered for the serious and even fatal bleeding which sometimes occurs in that condition. In addition, a fundamental point has emerged concerning the effect of age on fibrinolytic activity.

CHAPTER ONE

SURVEY OF PUBLISHED WORK ON FIBRINOLYSIS

HISTORY OF THE RECOGNITION OF FIBRINOLYSIS

"In many modes of destroying life the blood is deprived of its power of coagulation, as happens in sudden death produced by many kinds of fits, by anger, electricity or lightning: or by a blow in the stomach, etc. In these cases we find the blood, after death, not only in as fluid a state as in the living vessels, but it does not even coagulate when taken out of them"

John Hunter, 1794.

"---- great was my astonishment to find the fibrin had dissolved in most of the saline solutions"

P.S. Denis, 1838.
(translation)

The history of observation of fibrinolysis thus goes back a long time, but the history of the recognition of fibrinolysis as an important mechanism is modern. Macfarlane and Biggs (1948) can be given credit for initiating much of the present interest in the subject. In their review they made reference, in relatively short space, to most of the important discoveries of the last hundred years relating to fibrinolysis. These early observations were generally made at autopsy or as part of a laboratory type of investigation, and were not at first

recognised as having clinical importance. The name "fibrinolysis" came from Dastre (1893) who noted the disappearance of fibrin left in contact with the blood whence it had come and gave the phenomenon its new name - "C'est cette disparition de la fibrine que je nomme Fibrinolyse".

Morgagni (1769) described a case where death resulted from a stab wound in a man of 40 who had previously been healthy. When he dissected the body next day, his interest being in anatomy, Morgagni observed that the blood was "more dissolved and more watery than usual". In the posthumous work of John Hunter (1794) we find he recognised that the blood after sudden death was often incapable of clotting, and it can be assumed as a result of later work that he was observing the results of fibrinolysis. All along the history of fibrinolysis there has been a close association between demonstrated fibrinolysis and apparent fibrinogen depletion. Nolf (1905-06) reported that surgical removal of the liver in dogs led to fibrinolysis when this was estimated within half an hour of the operation. Fibrinogen deficiency followed. When post-mortem blood failed to clot Morawitz (1906) almost always found fibrinogen deficiency. He felt that the absence of fibrinogen was the result of fibrinolysis which could be so powerful as to remove all the fibrinogen and fibrin from the blood stream within ten hours of death.

An empirical observation was made by Denys and de Marbaix (1889) that the addition of chloroform, ether, alcohol or thymol was followed by a demonstrable proteolytic activity in the serum.

This was dependent on a factor which could be destroyed by heating.

Almost the first association of fibrinolysis with a clinical disorder diagnosed in life was made by Goodpasture (1914) who noted rapid dissolution of clots prepared from the blood of four patients suffering from atrophic hepatic cirrhosis. He also noticed that the potency of the lytic agent diminished with storage.

The discovery was made by Tillett and Garner (1933) that certain beta haemolytic streptococci, particularly those of human origin, create a substance in their culture medium capable of stimulating very rapid fibrinolysis when added to plasma clot and they called the substance fibrinolysin. Although this activity has been confirmed many times, it is still an observation without certain clinical relevance. Nevertheless great strides in the understanding of fibrinolysis have developed from the use of the product of the beta haemolytic streptococcus. The product has been shown to be inactive against clot prepared from purified fibrinogen, although the presence of euglobulin from normal human serum allows lysis to take place (Milstone, 1941). It was thus inferred that the agent was acting at an earlier stage of a more complicated system than had been realised and could no longer reasonably be called fibrinolysin. Christensen and MacLeod (1945) called the active exotoxin of the streptococcus, streptokinase.

Evidence about the existence of anti-fibrinolysis arose towards the end of the nineteenth century. Hahn in 1897 talked of the antifermentative action of the blood and Opie and Barker (1907)

quoted Landsteiner in 1900 as identifying antitrypsin activity in the albumin fraction of plasma. The proteolytic activity of chloroform-activated serum on gelatin and casein was demonstrated by Delezenne and Pozerski (1903 a, b). They then showed that this could be entirely neutralised by the addition of untreated serum. Hedin (1904) found that he could neutralize the digestive effect of his splenic alpha protease upon coagulated serum by adding albumin or, a little less effective, pseudoglobulin. The ferment-inhibiting action of serum was reported by Jobling and Petersen (1914) to be due to the presence of compounds of the unsaturated fatty acids, hence its removal by chloroform or ether, and a similar opinion was expressed by Ungar (1945) after using ether, although he felt that a water soluble fraction was also necessary for antitrypsin activity. Dale and Walpole (1916) accepted that chloroform destroyed the antiproteolytic potential of normal serum, as did Teale and Bach (1920) but they felt that their evidence favoured antitrypsin being protein in nature and not lipoidal. Christensen (1954) states that the inhibitor is not a lipid.

Subsequent work does not seem to have gone much further concerning the effect of chloroform on antiproteolysis, although it has been felt by some that the chloroform action is more complicated than can be accounted for by destruction of an inhibitor alone. Astrup (1956) thought that chloroform had a denaturing effect on one or more of the fibrinolytic factors to account for its vigorous activating effect.

Hussey and Northrop (1923) appeared to establish that serum inhibitor combines with trypsin in a stoicheiometric reversible

reaction but Shulman (1952) thought that the plasmin and inhibitor combination was irreversible. It is uncertain whether the difference between trypsin and plasmin is significant in this respect.

One of the most dramatic stories in the history of fibrinolysis is that of Yudin (1937) who used blood from the dead on a large scale for transfusion purposes, but only the blood from those who had died suddenly was suitably liquid (reliquefied) for his purpose. Severe trauma, such as operative surgery (Nolf, 1905-06: Macfarlane, 1937: Imperati, 1937) with cardiac arrest (Coon and Hodgson, 1952), peptone and anaphylactic shock (Rocha e Silva and Texeira, 1946: Rocha e Silva, Andrade and Texeira, 1946), electrically induced convulsions (Fantl and Simon, 1948), severe haemorrhagic shock and after extensive burns (Tagnon, Levenson, Davidson and Taylor, 1946) in man and in animals as well as sudden death, have all been associated with fibrinolysis. Macfarlane (1937) noted the relationship of fibrinolysis with actual stress on the body, and then with fear of stress and drew attention to this with Biggs (1946). Selye's concept of stress appeared appropriate to fibrinolysis but Truelove (1952) provided evidence against fibrinolysis being initiated by corticotrophin or adreno-cortical activity. Severe exercise also induced increased fibrinolysis (Biggs, Macfarlane and Pilling, 1947). The common factor in anxiety and severe exercise seemed to be adrenalin and this was in fact shown to cause intense fibrinolytic activity when injected experimentally into normal subjects. The short duration of adrenalin-induced fibrinolytic activity in the serum does not appear to be explainable by an increased excretion of such activity in the

urine (Williams, 1951: Macfarlane and Pilling, 1947). It has therefore been assumed that serum anti-plasmin neutralises this effect.

Another observation of Macfarlane (1937), made incidentally in the first place, has proved to have great investigational value. He found that dilution of plasma with saline led to a rate of lysis greater than that shown without dilution. This principle of rendering lysis apparent by dilution has been confirmed many times and has been used in many of the techniques since developed for the demonstration of fibrinolysis. The explanation of the dilution effect is still unproved. Certain exceptions to it will be demonstrated later.

Macfarlane's dilution technique demonstrated lysis frequently when it could not be shown in whole plasma, but it was usually found only in conditions of stress. Fearnley and Tweed (1953) modified the technique by manipulating the blood at ice temperature and were able to demonstrate lysis even in normal people at rest. Their experiments supplied evidence that there was a heat-labile fibrinolytic factor in normal plasma. This was not the first demonstration of fibrinolysis in healthy people at rest. Ratnoff (1949) had shown lysis in a high proportion of clots from healthy controls and ward patients by using recalcified plasma.

Several recent advances in knowledge about fibrinolysis have arisen from the use of plasma fractionation. This technique is not a new one. Hedin (1904) used ammonium sulphate fractionation, one third saturation with ammonium sulphate of dilute ox serum

bringing down euglobulin, one third to one half saturation bringing down pseudoglobulin, and that appearing between half and full saturation, albumin. These fractions were tried out for their respective powers of digestion. Opie and Barker (1907) also used salt precipitation. Feissly (1942) and Macfarlane and Pilling (1946) used acid precipitation. The most important lytic agent seems to be present in the globulin fraction, and euglobulin fractionation (Milstone, 1941: Sherry, Lindemeyer, Fletcher and Alkjaersig, 1959) is one of the most successful of current techniques for the demonstration of fibrinolysis. Wilder and Parsons (1956) and Astrup, Piper and Rasmussen (1960) have each demonstrated normal fibrinolysis in a case of agammaglobulinaemia and the latter group in a case of macroglobulinaemia. This appears to dissociate the euglobulin fibrinolytic activity from the gamma- and macro-globulin moieties, but at the moment there may be more evidence against such a dissociation than there is for it. There may well be more than one fibrinolytic agent in any one fraction, but the process of fractionation helps to separate the lytic agents from anti-lytic factors and in particular separates off the anti-fibrinolytic or antiplasmin agents, these being variously ascribed to the albumin fraction, the alpha 1 and the alpha 2 globulins. Antifibrinolytic factors are discussed in the appropriate section.

Important aid to the further analysis of fibrinolytic mechanisms came from the fibrin plate technique of Permin (1950). This gave more positive evidence of there being an intermediate step in fibrinolysis by demonstrating a difference between human

and bovine clot. Streptokinase did not lyse purified fibrin from any source, nor did it lyse untreated bovine fibrin clot; but it did lyse untreated human clot. If a little human serum was added to the bovine fibrin, then streptokinase became actively fibrinolytic there also. It was deduced that human blood contains a substance not present in bovine blood which acts as an intermediary between streptokinase and plasminogen. The intermediate factor which streptokinase converts into activator was called proactivator, and it appeared that plasminogen and this proactivator (of plasminogen) were both adsorbed on to freshly formed fibrin. Comment is made later concerning this work in its relationship to the nature of proactivator.

Heating a fibrin plate for about half an hour to approximately 80 degrees centigrade (^{o}C) was found to inactivate the plasminogen and proactivator and so the fibrin became susceptible only to trypsin-like lytic agents such as plasmin itself (Lassen, 1952). Early fibrinolytic components were incapable of lysing the fibrin by themselves and retained this power only after first activating intermediate factors. These could be added selectively and their action thus studied. Heated and unheated fibrin plates used together have contributed greatly to an understanding of some of the mechanisms involved in fibrinolysis.

Tissue substances have been shown to have an influence on the fibrinolytic system and Astrup and his colleagues have been responsible for a very large proportion of the work in this field (Astrup, 1956). A relatively insoluble agent found in the tissues

acts directly on plasminogen. This agent was called fibrinokinase at first, but the name was later changed by Astrup to tissue activator.

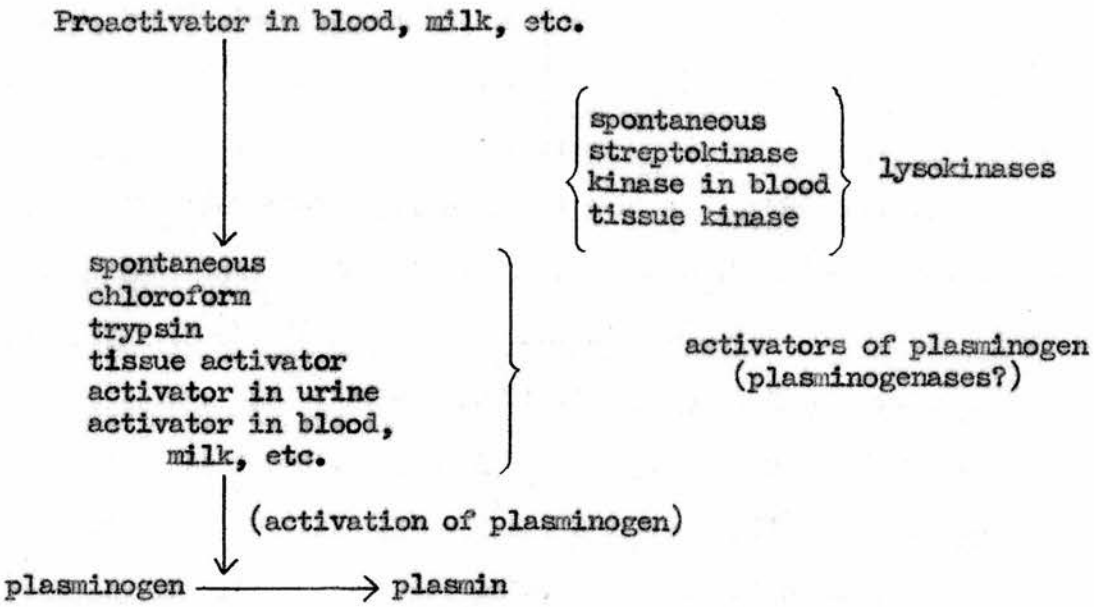
An interesting group of observations arose from studies on the fibrinolytic effect of normal urine. Farnsworth, Speer and Alt (1946) quoted Brücke in 1861 as being perhaps the first to draw attention to the presence of a proteolytic substance in urine. They then sketched the subsequent history of this observation but themselves believed the protease to be pepsin or pepsinogen. Macfarlane and Pilling (1947) and Williams (1951) referred to the activity in urine as fibrinolytic. This fibrinolytic factor in urine can be shown to be a water soluble activator of plasminogen which is relatively heat stable at neutral and alkaline reaction (Astrup and Sterndorff, 1952). Other body fluids secreted through narrow channels were then investigated and it was found that human milk (Astrup and Sterndorff, 1953), "emotional" tears (Storm, 1955), saliva (Albrechtsen and Hess Thaysen, 1955) and seminal fluid (von Kaulla and Shettles, 1953: Lundquist, Thorsteinsson and Buus, 1955) all contained large quantities of proactivator, some activator and usually no plasminogen. Plasma differed in having plasminogen in addition. It is assumed that this concentration of early fibrinolytic factors has the function of keeping the small ducts clear of obstruction from unwanted clot (which would bring adsorbed plasminogen with it). Most tissues of the body contain a fibrinolytic substance or substances which can be demonstrated after saline extraction but there is some confusion about the interpretation of the mechanisms involved. Müllertz (1955) kept

many of these observations together by his analysis of the lytic components, all of which acted through the common pathway of plasminogen.

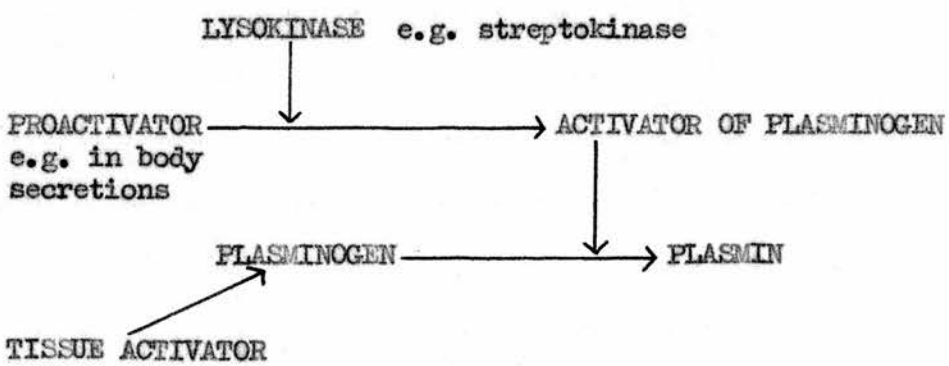
Kwaan, Lai and McFadzean (1960) claimed to be the first to demonstrate unequivocally the presence in a body fluid of a lysokinase, having activity at the same level as streptokinase, but being a naturally occurring agent it was of greater pathological or physiological significance. They used ascitic fluid from hepatocarcinoma and believed the active fraction to be in the gamma globulin while the beta globulin fraction had antilysokinase activity.

Tissue lysokinase activity, provided it has been correctly interpreted, appears to have been recognised for some time. Fantl and Fitzpatrick (1950) showed that brain extract prepared as for the Quick test is able to act with a serum factor to form a lysin of unheated but not of heated fibrin. Astrup and Sterndorff (1956) failed to find any lysokinase activity in human lung, liver or adrenal and found it only erratically in kidney and lymph glands.

It has been widely accepted, although often questioned, that streptokinase, generically a lysokinase, is active only on the proactivator of plasminogen which it converts stoicheiometrically (Müllertz, 1955) to the activator. This then changes plasminogen catalytically to plasmin. The following is the scheme of fibrinolysis presented by Astrup in 1956.



Simplified form of fibrinolytic scheme: The Astrup fibrinolytic scheme of 1956 showing the interrelationship of the various fibrinolytic factors has been reproduced above. Even now the presentation seems too detailed for a subject which is still so full of technical and interpretative pitfalls. A simpler scheme which remains based on the Astrup figure seems more acceptable and less confusing.



This scheme bears a superficial resemblance to the currently accepted coagulation scheme, where tissue juice can by-pass the more complicated coagulation mechanism of the blood.

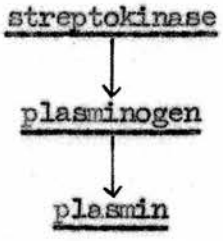
It should be noted that the Astrup scheme is characterised by the separate existence of proactivator. Further comment about the existence of proactivator is made later in the appropriate section, together with a revised scheme of fibrinolysis.

Various successful experiments have been carried out *in vivo* to assess the therapeutic effect of fibrinolytic agents. Cliffton, Grossi and Cannamela (1953) injected human plasmin into three species of animal, with rapid lysis of intravascular clots. Some of the investigations which have been carried out more recently are described in the section devoted to experimental methods of increasing fibrinolysis ("thrombolytic activity").

NOMENCLATURE

The obvious name for the most important lytic agent of fibrin appears to be "fibrinolysin", but Christensen and MacLeod (1945), while using the word themselves, criticised its use because it did not embrace the accepted ability of the enzyme to lyse proteins other than fibrin. Macfarlane and Biggs (1948) also objected to its use. They pointed to the confusion which existed already from the use of the term fibrinolysin to describe the bacterial agent of the beta-haemolytic streptococcus.

It is proposed to use the nomenclature suggested by Christensen and MacLeod with additions. This nomenclature is logically derived, although it is perhaps insufficiently descriptive for those unfamiliar with the subject. It is based on the analogy of certain pancreatic proteases. The active agent is called plasmin, where the prefix indicates the plasma source of the enzyme, the word as a whole conforming with common usage for proteases. The inactive precursor of plasmin is called plasminogen, the zymogen of plasmin, and the active principle in streptococcal filtrate, streptokinase.



Nomenclature of Christensen and MacLeod (1945).

This terminology has been followed very widely since it was first proposed. New additions to the nomenclature have become necessary as the fibrinolytic system has been found to be more complicated. These additions have tended to be based on the names put forward by Christensen and MacLeod, and have been self-explanatory, such as "activator" and "proactivator" of plasminogen, "staphylokinase" (Lewis and Ferguson, 1951) and others. Some such names have been superimposed on the original nomenclature below.

lysokinase or fibrinolysokinase e.g. streptokinase, staphylokinase

↓

proactivator of plasminogen

↓

activator of plasminogen

↓

plasminogen: syn. profibrinolysin.

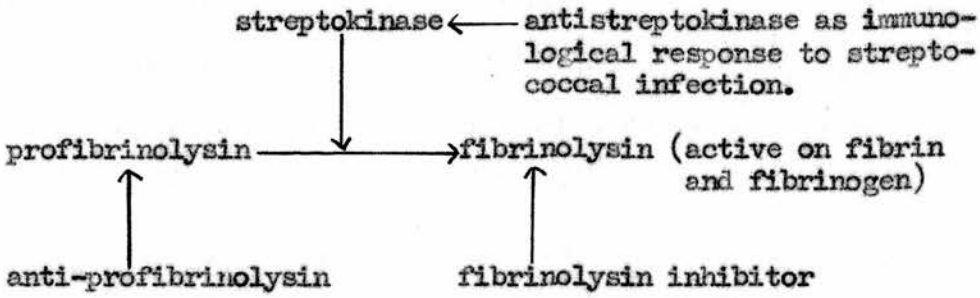
↓

plasmin: syn. fibrinolysin: serum protease

The original nomenclature has been underlined.

Activators of the proactivator have been given the generic name of lysokinase (a shortened version of fibrinolysokinase) in order to show their group relationship to streptokinase, the best known and longest recognised constituent member. When Astrup and Permin (1947) described the tissue activator substance, they called it fibrinokinase at first, believing it to be similar to streptokinase. They expressed their opinion that streptokinase itself was imperfectly named. They suggested that the whole group to which streptokinase belonged should really be called fibrinokinase, that their tissue activator might be called cyto-fibrinokinase and that streptokinase itself should be called strepto-fibrinokinase. Etymologically they seemed correct at the time, but had to drop the names when it became apparent that the two substances did not in fact belong to the same group. It is easy to see, therefore, how the literature on fibrinolysis has become studded with confusing nomenclature.

Loomis, George and Ryder (1947) proposed the following nomenclature:



The classification does not appear in 1960 to justify the criticism passed on it by Macfarlane and Biggs in 1948, when they described the use of the word "fibrinolysin" for plasmin as fraught with danger because the streptococcal factor had been so named previously. Astrup (1956) recognised both classifications but expressed a preference for the one based on "plasmin" rather than "fibrinolysin" for the same reasons as Macfarlane and Biggs.

Although it is tempting to talk of profibrinolysin instead of plasminogen, there seem to be two good reasons for not changing to the classification of Loomis, George and Ryder at this stage. Firstly there is still far too much uncertainty whether or not more than one lytic agent exists in plasma; and secondly the more changes there are in nomenclature, the greater the confusion.

There seems to be a place for the free use of the word fibrinolysin, or its plural fibrinolysins. These would mean any fibrin-lysing agent in the blood, whether identifiable as plasmin or not.

There also seems to be a place in clinical practice and in related laboratory work for the word thrombolysis to describe the process of dissolution of a specific clot forming a thrombus, but the active agent should remain as plasmin or fibrinolysin. There seems no place for the word thrombolysin.

Sherry, Fletcher and Alkjaersig (1959) recommended a different use of the word thrombolysis. They felt that a distinction should be drawn between the lysis of a thrombus or other fibrinous material, for which they would use the word thrombolysis, and the lysis of purified fibrin which they felt was only part of the former process and alone justified the word fibrinolysis. It seems debatable whether it is profitable or even possible to impose a slightly different meaning on to a word already in general use, without increasing the confusion in nomenclature.

Other names have been suggested for components of the fibrinolytic system, but have not achieved general acceptance. Clumsy words like serum proteaseogen and fibrinolysinogen are as well forgotten.

THE COMPONENTS OF THE PLASMIN OR FIBRINOLYTIC SYSTEM

None of the components of the fibrinolytic system has been identified chemically. Like the blood coagulation system, the presence of each component has had to be inferred from the results of leading a series of reactions through to the end product which is plasmin itself. Fibrinolytic activity so

derived is the only indicator of the presence of plasmin precursors and of activity within the system.

Even although plasmin itself is the end product towards which the whole fibrinolytic system is geared, its presence can only be deduced from the effect on an appropriate protein which must be added to the test material if it is not already present. Such protein, of which fibrin is the most important example, is not an essential part of the fibrinolytic system, but is necessary as an indicator of its activity. It adds another variable to investigational work. In laboratory experiment the plasmin system is inferior to the coagulation system in respect of its indicator, for the latter supplies its own.

Some of the components of the fibrinolytic system will be described individually. They will be taken in reverse order of their believed action, starting with the protease itself and working backwards. The various agents are those of a simplified Astrup classification, but it will become clear that some such divisions are of doubtful justification. Indeed, two sections are devoted to two components which may be one and the same.

Plasmin

Plasmin itself behaves as a proteolytic enzyme active at neutral pH. It is moderately unstable and this has hampered its isolation in relatively pure form, although its increased stability at pH 2 has proved useful in attempts to purify it (Kline and Fishman, 1957). Recent progress in the purification

of its precursor, plasminogen, has allowed the preparation of plasmin of superior specific activity. Such plasminogen can be activated spontaneously or by the use of a specific activator such as streptokinase. Plasmin is destroyed by heating to 55°C for twenty minutes. Its molecular weight has been calculated to be 108,000 (Shulman, Alkjaersig and Sherry, 1958). Its action is not restricted to the lysis of fibrin, although it appears to have a relatively more powerful action against fibrin than have other proteolytic enzymes. Tests for its presence may use casein, gelatin or heated fibrin with apparently equal success and other proteins or synthetic substrates can be used. The ability of plasmin to split arginine and lysine esters and the discovery that the latter act as competitive inhibitors of plasminogen activation, promise to be of assistance in the understanding of how plasmin works, although the ability to split an ester is no proof of fibrinolysis. Plasmin can also digest all the proteins of normal plasma including early coagulation factors such as factor V and prothrombin and part of complement. Plasmin showing such activity has usually been prepared artificially by adding streptokinase or chloroform.

Plasmin is apparently fibrinogenolytic (Keckwick, Mackay, Nance and Record, 1955) but here a point of controversy has arisen. Mole (1948) and Bidwell (1953) showed the presence of normal fibrinogen levels despite active fibrinolysis in cadaver blood and after exercise. Jacobsson (1955) also found no fibrinogenolysis in fibrinolytically active plasma following

exercise. Kwaan, McFadzean and Cook (1956) showed that the fibrinolysin present in cirrhosis of the liver had no effect on fibrinogen, prothrombin, gelatin or casein and therefore in their opinion was not plasmin. Biezenski and Moore (1959) reported that the fibrinolysin demonstrable after obstetrical accidental haemorrhage, and before delivery, was fibrinolytic but not fibrinogenolytic. Thus, proven fibrinogenolysis has usually been demonstrated only after artificially stimulated lysis and not in naturally occurring states.

Such evidence may be interpreted as favouring the existence of at least two fibrinolysins, one being what is generally described as plasmin, a fibrinogenolytic enzyme which is usually artificially produced in plasma by streptokinase or chloroform, and another, a less well identified enzyme which is not fibrinogenolytic, but which is more commonly described in naturally occurring fibrinolysis. Thus plasmin may not be the only lytic agent which can appear in the blood. Indeed plasmin itself is not necessarily a single substance, even within its own definition, despite attempts to purify it and to define it biologically. Ungar, Damgaard and Hummel (1953) suggested that the word plasmin designated several different enzymes.

On the other hand and not necessarily in conflict with such ideas, it remains possible that fibrinolysis is always fibrinogenolytic but that this is not apparent because inhibitors deny the lysin any access to circulating fibrinogen (Müllertz, 1953). When fibrin is formed, plasminogen and activator are selectively adsorbed on to the clot. Thus the agent which was

inhibited in its action on circulating fibrinogen is free to act upon fibrin. Such an inhibitor may act also on the activator of plasminogen as well as on plasmin.

Plasmin appears to act by degrading fibrin and other proteins into large polypeptides and it releases arginine and lysine after digesting fibrinogen (Cohen, 1959). Proteins which are split in the process of lysis have been shown to have electrophoretic mobilities of alpha, beta and gamma globulins (Kowalski, Budzyński, Kopeč and Murawski, 1960).

Less attention has been paid recently to the great similarity between trypsin and plasmin, but it has been claimed all along by some and is now accepted generally that they are not one and the same enzyme. Denys and de Marbaix (1889) and later Kaplan (1946) drew attention to differences between trypsin and plasmin. Cliffton and Cannamela (1953) noted that the fibrinolytic activity of plasmin and the proteolytic activity of trypsin were not inhibited by equivalent amounts of soya bean inhibitor. Sherry, Fletcher and Alkjaersig (1959) list the points of difference between trypsin and plasmin as follows:

1. trypsinogen and plasminogen require different activators;
2. plasmin and trypsin have different ratios of attack upon synthetic substrates;
3. the inhibition of these enzymes by crystalline soya bean inhibitor and organophosphorus compounds is different;
4. casein digested by plasmin may be further hydrolysed by trypsin, but the reverse does not obtain.

When uninhibited, plasmin activity in the blood stream is very powerful and is almost certainly greater than is ever called upon in the maintenance of health. Massive release of this huge reserve of plasmin has been suggested as an explanation of anaphylactic shock. Ungar and his colleagues (1953) noted the symptoms and tissue changes associated with anaphylaxis when they injected into animals peptone and high molecular weight carbohydrates which induced proteolytic activity. There was a high correlation between proteolysis and histamine release, the proteolysis probably coming first.

Plasminogen.

The immediate precursor of plasmin is plasminogen, which behaves as part of the globulin fraction III (Cohn) of plasma and may be a glycoprotein (Sherry, Fletcher and Alkjaersig, 1959). It is converted to plasmin by the catalytic activity of plasminogen activator (Müllertz, 1955). Plasminogen appears to have affinities with fibrinogen with which it is brought out of solution unless special precautions are taken (Remmert and Cohen, 1949: Keckwick, Mackay, Nance and Record, 1955). In vivo exudates and transudates which have a low fibrinogen content tend to have a low plasminogen content: where fibrinogen is abundant plasminogen is present in high concentration (Christensen, 1954). Plasminogen can be brought out of solution by the Milstone (1941) technique of dilution twenty times with water and 0.32 volumes of 1 per cent acetic acid, and further purification can be made by isoelectric precipitation (Remmert and Cohen, 1949): acid

extraction, making use of the important observation that plasminogen is soluble in dilute mineral acid (Christensen and Smith, 1950): or by the method of Kline (1953) who claimed an increase of purity of 425-fold as compared with serum. He also claimed to obtain crystalline plasminogen. Heat stability varies with pH, being greatest about pH 2 (Sherry, 1954). The physical properties of highly purified human plasminogen have been described by Davies and Englert (1960). They made observations on the sedimentation constant, diffusion constant, partial specific volume, intrinsic viscosity and electrophoretic mobility. They calculated the molecular weight as 83,800. Shulman, Alkjaersig and Sherry (1958), in a similar type of investigation, found the molecular weight of plasminogen to be 143,000. One of the most interesting observations of Davies and Englert, to be taken with those of Hagan, Ablondi and de Renzo (1960), is their failure to differentiate plasminogen from proactivator of plasminogen. The two appeared to be the same substance, although there is no question as yet of their identity being proved. They admit to their purified plasminogen having at least two antigenic actions.

Plasminogen becomes adsorbed to fibrin clots formed from whole plasma, both in man and in cattle. If clots are to be freed from plasminogen, the latter can be destroyed by heating the clots to about 80°C for about half an hour. This appears to leave fibrin itself unchanged, although some less obvious alteration seems probable. Considerable latitude is allowed in time and temperature in the process of deliberately inactivating

plasminogen without obviously affecting the result. When plasminogen is activated to plasmin, the same amino-acids are released as appear when plasmin digests fibrinogen, suggesting a similar enzymatic process (Cohen, 1959).

If it is wished to draw a very rough parallel between the fibrinolytic system and the coagulation system, plasminogen can be likened to fibrinogen; activator of plasminogen to thrombin; and plasmin to fibrin.

Activators of Plasminogen.

Tissue activator: Astrup and Permin (1947) and Permin (1947) showed the existence of a relatively insoluble direct activator of plasminogen, present in a great variety of tissues. At first they called this agent fibrinokinase, but later changed its name to tissue activator. Tissue activator is stable and is insoluble except in concentrated potassium thiocyanide, and has been shown to be present in large amounts in the uterus, adrenal, lymph nodes, prostate, thyroid, lungs and ovary. Smaller amounts are present in the pituitary, kidney, muscles, heart, testes and spleen. Liver tissue is almost inactive (Albrechtsen, 1957). Human erythrocyte stroma has been shown to be a very active source of tissue activator (Permin, 1947). The tissue activator is a particulate component of these tissues and is probably contained in the microsome fraction (Lewis and Ferguson, 1950b).

Fischer (1946) may have been the first to demonstrate tissue activator present in growing tissue cultures. He talked

unequivocally about its species specificity and referred to his own report of 1927 on the subject. He wrote, "In other words tumour cells seem to be able by contact to activate the proteolytic proenzymes in the homologous blood-plasma. Our experiments show that the activation is rather specific." Barnett and Baron (1959) and Baron and Barnett (1960) supplied good evidence that tissue activator is created in some tissue cultures used in the preparation of poliomyelitis vaccine. They made the interesting observation that more activator could be demonstrated in the medium than could ever be demonstrated in the cells at any one time, suggesting a metabolic production and then a release of the activator into the medium.

Tissue activator does not appear to have been successfully freed from impurities to allow of more exact characterisation, although partial purification was claimed by Astrup and Sterndorff (1956a) using pig heart and lung, and further success ought to follow the demonstration of tissue activator in tissue culture. Tissue activator activates plasminogen independently of streptokinase and it does not appear to substitute for streptokinase in an appropriate experimental system. In these and in other ways the tissue activator seems quite distinct from the soluble proactivator/activator which is found so freely in body fluids.

Labile activator: A more labile activator of plasminogen is present in the blood, but it is usually only

demonstrable in such conditions as have been referred to already, associated with increased fibrinolysis. Müllertz (1956) believes that there is a constant trace level of the labile activator in the blood. This labile activator of plasminogen comes out of solution with the euglobulins of plasma and is adsorbed to fibrin in the course of clotting and is relatively easily destroyed by heat; but it has not been successfully isolated and this has hindered a study of its relationship to the disputed proactivator, to the tissue activator and to plasmin itself.

The content of labile activator in those secretions which have to pass through narrow channels - milk, tears, saliva, seminal fluid - has already been referred to in the section devoted to the history of fibrinolysis. It has been claimed that activator is present in these secretions but less abundantly than proactivator. The activator which can be demonstrated in urine justifies further comment, if only because of the amount of work which has been done upon it. It is less certain that this justifies continuing with the individual name of urokinase which was initiated by Sobel, Mohler, Jones, Dowdy and Guest (1952).

Urinary activator: The urinary activator of plasminogen was probably the first physiological activator of plasminogen to be isolated in a highly purified form. It can be concentrated by adsorption and elution from barium sulphate and by more complicated but essentially similar methods. By these means it appears to have been purified to a fairly high specific activity. Electrophoresis suggests that the urinary

activator accounts for about 37 per cent of the urinary protein prepared as "urokinase" (Ploug and Kjeldgaard, 1957). It is colourless and stable over a wide pH range unless in acid urine when it is rapidly destroyed. It appears to transform plasminogen to plasmin by a first order enzyme reaction (Kjeldgaard and Ploug, 1957) and at great speed (Alkjaersig, Fletcher and Sherry, 1958b). However, in high concentrations, as have been possible by purification methods, the agent seems to be proteolytic and to resemble plasmin itself, particularly in its ability to split the two amino-acid esters of arginine and lysine, to which reference has already been made (Kjeldgaard and Ploug, 1957). It seems possible to readers of the subject that this urinary activator could be plasmin itself, bound perhaps to a stabilizing factor which alone renders it different from plasmin as it is at present recognised.

Kolmen, Guest and Celander (1959) presented good evidence that red cells can adsorb urokinase, where urokinase is defined as a saline soluble agent in urine, acting directly on plasma profibrinolysin (plasminogen) to convert it to fibrinolysin (plasmin). They then proposed a rather complicated system of regulation of fibrinolysis by changes in the amount of lytic agent and inhibitors adsorbed on to red cells. It seems unlikely that the red cells can be responsible for such a finely adjusted control over fibrinolysis by virtue of their passive powers of adsorption, when one considers the apparently invariable occurrence of delayed lysis in whole blood clots as compared with plasma clots.

Plasmin has been credited with some activator activity, which can be demonstrated in a system of purified factors. This would be a feed-back mechanism. Thus, plasminogen can be shown to undergo "spontaneous" activation around pH 7 when 50 per cent glycerol is added to a purified preparation, glycerol stabilising both plasminogen and plasmin. The yield of plasmin however is produced slowly and continues for four to nine days (Alkjaersig, Fletcher and Sherry, 1958a). This is evidence in favour of autocatalytic activity, the glycerol acting as a passive stabilizer. Plasmin was shown to accelerate this reaction, behaving in this context as an activator. However, it remained a slow reaction which was susceptible to plasmin inhibitors when present and to destruction from the general instability of its parts. Such evidence draws attention again to the similarity in effect of plasmin and the labile activator of plasminogen.

Proactivator of plasminogen.

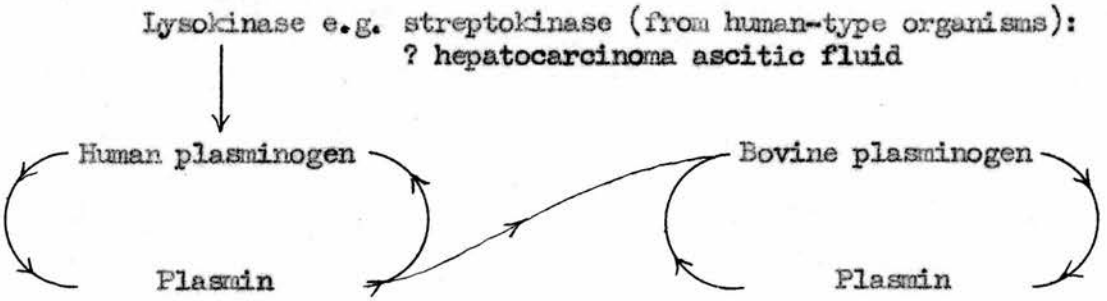
While there is no doubt about the existence of activator of plasminogen, there is much current debate about the existence of proactivator. Until recently the evidence seemed very convincing in support of the existence of a distinct proactivator of plasminogen. The fibrin plate technique of Permin (1950) and the heated fibrin plate modification of Lassen (1952) appeared

to be clear cut research tools which proved the point. Streptokinase was inactive against heated fibrin from any source. It was also inactive against unheated bovine fibrin which was known to be rich in plasminogen; but was very active against unheated human fibrin. If a little human serum was added with the streptokinase, to unheated bovine fibrin, then the latter was lysed rapidly. Clearly bovine plasma lacked the intermediate agent which was present in human plasma; this agent was called proactivator. There is, however, another interpretation of this which will be discussed below. Proactivator was then characterised as a globulin which came out of solution with fibrinogen and by adsorption, with fibrin. In these respects it was like plasminogen. It was converted to activator by streptokinase.

Tillett (1938) however had shown that some bovine streptococcal exotoxins did in fact lyse bovine clot. Perhaps proactivator is itself species specific and is present in bovine plasma. Perhaps the present state of uncertainty has arisen from an almost exclusive use of commercially prepared human-type streptokinase. At any rate, the experiments quoted above do not themselves appear to prove the existence of a cofactor with quite such certainty as at first appeared. Some cofactor does appear to be necessary when streptokinase originating from a human-type streptococcus is required to activate plasminogen of another species. In the classic experiment above, human-type streptokinase requires a cofactor in order to activate bovine plasminogen.

Reconsideration of the whole situation brings attention to the fact that without the streptococcus, pro-activator might hardly be thought to exist. Proof is also lacking that the exotoxin of the streptococcus, a substance foreign to the body, is reproducing a physiological reaction at all. Only the products of certain strains of streptococci are active plasmin inducers in human plasma. They may be inactive in others. Tillett (1938) made comment on this apparent predilection of strains of haemolytic streptococci for the fibrin of the same species as that in which the organism may survive. Thus an active human-type streptokinase is not active against bovine clot: equine streptokinase, active against horse clot, is not active against human clot: and as already stated, active bovine streptokinase is sometimes lytic against bovine clot. It remains possible that streptokinase is an unphysiological enzyme, specific only for certain plasminogens, usually those of the same species as the original host. The plasminogen, being activated, releases plasmin which, however, may not be species specific and may perhaps activate such plasminogen as bovine plasminogen which is otherwise insusceptible to that particular streptokinase. The species specificity of the streptococcal exotoxin weakens greatly the argument in favour of there being an independent proactivator.

A simplified scheme put forward to explain the action of streptokinase without incurring the existence of independent proactivator is presented below.



It seems improbable that the simplified scheme presented here can be the whole answer. Hagan, Ablondi and de Renzo (1960) declare it to be highly unlikely that plasmin can account for all the activator activity and cite investigations showing that a mixture of streptokinase and plasminogen containing a large concentration of plasmin, or trypsin activated plasmin, is at best a poor activator of bovine plasminogen.

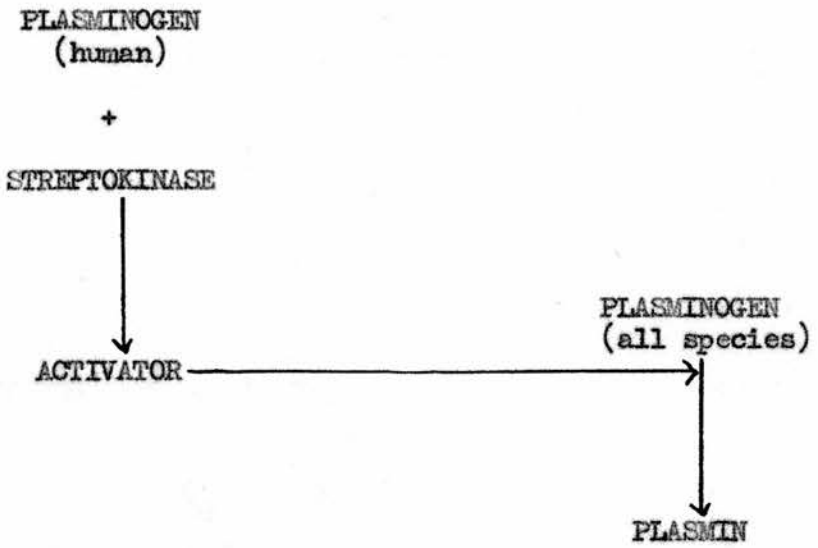
While Astrup's experiments with fibrin plates seemed to prove the need for a proactivator which is present in human blood but lacking in other species, Kline and Fishman (1957) interpreted the same experiments differently. They felt that their own views were in harmony with the experiments of Sherry (1954). They explained the results from both laboratories in terms of streptokinase requiring human plasminogen to convert it into a "universal activating enzyme" which then activates the plasminogen of any species. It may be observed at this stage that species specificity still exists according to this theory. Perhaps bovine-type streptokinase requires bovine plasminogen and then it too will become an enzyme capable

of acting on the plasminogen of other species. Such an experiment, important as it is in the theoretical pattern, does not appear to have been tackled.

Kline and Fishman (1957) prepared a purified system involving plasminogen-free human fibrinogen, plasminogen-free bovine thrombin and as pure a streptokinase as was available and they labelled some of the streptokinase with ^{131}I and then tested their preparations for plasminogen content. They supplied evidence that proactivator and plasminogen were identical substances. By using ^{131}I-labelled streptokinase they showed quantitative utilisation of the radioiodine in the part which they subsequently isolated by alcohol fractionation. This implied incorporation of streptokinase into either activator or plasmin, both of which come down in the alcohol fraction. Purification of the plasmin component of the fraction led to loss of radioactivity by which it was inferred that streptokinase was incorporated quantitatively into the universal activator itself (there to lose its specificity).

In the results of Kline and Fishman it was apparent that proactivator was present in fibrinogen preparations which were plasminogen-free. How then could proactivator and plasminogen be one and the same substance? They explained this apparent obstacle to their theory by regarding the first reaction with streptokinase as requiring only minute amounts of plasminogen for a stoicheiometric reaction, but the second part

as requiring relatively massive amounts of plasminogen which are converted catalytically to plasmin. Their theory has much to commend it. It is unusual in its claim to simplify rather than complicate previous schemes of fibrinolysis. The following is a diagramatic representation of the scheme of Kline and Fishman (1957).



This and later work has supported the concept that proactivator and plasminogen activities are not entirely distinct and in fact reside in the same molecule. Davies and Englert (1960) found the same proportion of proactivator to plasminogen in highly purified plasminogen as they found in plasma or crude fraction III.

Until recently there was no body substance which appeared to substitute for streptokinase in the fibrinolytic system. This gives point to criticism levelled against the streptokinase effect as being unphysiological and perhaps not

even pathological. However Kwaan, Lai and McFadzean (1960) published a preliminary report claiming to demonstrate the presence of lysokinase activity in the ascitic fluid of seven patients suffering from carcinoma of the liver. It is too soon to know with what weight to use this finding as an argument in favour of there being an independent proactivator of plasminogen.

Kowalski, Kopec, Latallo, Roszowski and Sendys (1958) claimed to show a plasminogen-like proenzyme in human tissues, particularly from those organs rich in connective tissue such as aorta, veins and from fascia. They claimed that this plasminogen-like substance could be activated by streptokinase. There are certain ambiguities about the descriptions of method and controls, but it is possible if one follows the scheme of Astrup (1956) to describe their substance as proactivator of plasminogen. On the other hand, if an independent proactivator does <u>not</u> exist, the authors' claim to have demonstrated a plasminogen-like proenzyme can be supported - particularly since they had some success dissolving their substance in dilute mineral acid, which is a property of plasminogen.

<u>Lysokinase</u>

If one accepts the existence of a separate proactivator, then the formation of soluble activator itself is easily explained. A lysokinase converts proactivator to activator (Müllertz and Lassen, 1953). Most experimental work has used streptokinase, which is a lysokinase and is the stimulating factor which has set

the pattern for this early stage of the fibrinolytic system. Fibrinolytic activation of human plasma by the streptokinase which is prepared commercially is relatively violent and is not obviously inhibited by the anti-plasmins of the blood. Not all the details of the preparation of commercial streptokinase are known. Streptokinase appears to be a protein (Christensen, 1954) and was shown by Lassen (1958) to be strongly adsorbed on to glass surfaces.

There seems to be agreement that streptokinase activates human plasminogen in a two step process in which the first step is stoicheiometric and the second step a first order reaction (Sherry, Fletcher and Alkjaersig, 1959). In other words the existence of some sort of a proactivator appears to be accepted, whether proactivator is, or is not, a substance distinct from plasminogen itself. Nevertheless, it should perhaps be said once again that streptokinase is not a naturally occurring substance (unless in the invasive phase of a streptococcal infection) and caution must be used before assuming that it mimics a naturally occurring process.

Streptokinase alone will not hydrolyse lysine and arginine esters, but Alkjaersig, Fletcher and Sherry (1958b) showed that these substances act as competitive inhibitors of the activation of fibrinolysis as stimulated by streptokinase. Without pressing the point, they do mention that it is possible to argue from the evidence that the cofactor required for the activation by streptokinase, is plasmin.

In 1950 Fantl and Fitzpatrick described a property of human adult brain which can be interpreted as lysokinase activity of tissue origin. The factor was extracted by salt solution and was present in the globulin fraction. It was species specific and not identical with thromboplastin. The enzyme produced by the combination of plasma with the brain factor breaks down fibrin made by clotting procedures but shows no action on plasma fibrinogen or heat inactivated fibrin. It is possible to interpret their experiments in two ways. They assumed that the lytic enzyme, resulting from the brain and plasma combination, failed to act on heated fibrin because the fibrin was denatured. If, however, one assumes that the "heat denatured fibrin" is comparable to the heated fibrin plates of Lassen (1952), then the failure to lyse in that particular experiment could be attributed to the destruction by heat of plasminogen adsorbed to the clot. Thus the brain extract would be acting as a lysokinase, comparable to streptokinase, and would be activating proactivator in plasma. The brain extract of Fantl and Fitzpatrick (1950) is not likely to be the same as the tissue activator found so widely in the body by Astrup and Permin (1947) because of its solubility in salt solution.

Not until recently has it been claimed that a body fluid has lysokinase activity and in the case of Kwaan, Lai and McFadzean (1960) this was ascitic fluid from hepatocarcinoma arising after cirrhosis.

On the whole it is not felt that there is yet a case

which is unassailable for the existence of a naturally occurring physiological lysokinase.

Inhibitors of fibrinolysis.

It has often been assumed that anti-plasmin activity is synonymous with anti-trypsin activity, but there is "abundant" evidence that this approach is unsatifactory and that anti-tryptic and anti-plasmin activities may vary independently in biological fluids (Sherry, Fletcher and Alkjaersig, 1959). These authors also supply references to the list of synthetic inhibitors given later in this section.

There is no clear evidence defining the number of agents in the blood which are inhibitory to fibrinolysis, but it is reasonably certain that at least one inhibitor is an anti-plasmin. It is not clear, because of technical difficulties, whether an anti-activator exists. Epsilon-aminocaproic acid may be one such (Nilsson, Sjoerdsma and Waldenström, 1960).

Milstone (1941) found anti-plasmin activity in the pseudoglobulin fraction and Macfarlane and Pilling (1946) used the supernatant after precipitating euglobulin, calling this albumin. Others have found plasmin inhibitors in relation to Cohn's fractions. Dausset, Bergerot-Blondel and Colin (1956) found that dilution of plasma with saline speeded fibrinolysis more than dilution with fresh serum, unless that serum had first been inactivated by heat. They suggested that this result could be explained by destruction of an anti-plasmin by heat.

It seems that anti-plasmin activity is found mainly in

the alpha-2 globulin fraction (Jacobsson, 1955) but also perhaps in the alpha-1 fraction (Norman and Hill, 1958) although Jacobsson thought that alpha-1 activity was mainly anti-trypsin and not anti-plasmin. Norman and Hill used the lysis of casein as their indicator and so were measuring proteolytic activity rather than fibrinolytic activity as such. As noted in the historical section, anti-fibrinolytic activity has been associated from time to time with a fat component of plasma. Kwaan, Lai and McFadzean (1960) felt that the anti-fibrinolytic activity of their ascitic fluid was due to a lipoprotein.

Ratnoff, Lepow and Pillemer (1954) thought there might be at least three plasmin inhibitors, although this view is unconfirmed. If there are two agents, as many seem to think, the general feeling is that one reacts quickly with plasmin and the other slowly. Hussey and Northrop (1923) reported that the plasma inhibitive agent combined with trypsin to form an inactive but dissociable compound. With the evidence about anti-plasmin so uncertain, there has been no opportunity to study its total potential, although claims have been made that total anti-plasmin activity of the blood at least exceeds total plasmin potential. An anti-fibrinolysin fraction is presumably what is dissolved away or destroyed by chloroform and ether, but if the chloroform is used in a certain way there is also rapid destruction of active lysin (Lewis and Ferguson, 1950a).

Ungar and Damgaard (1951) showed that the anti-fibrinolytic activity of serum in guinea pigs and rats is

significantly increased by corticotrophin or cortisone. No such increase was obtained in splenectomized guinea pigs. This led to observations identifying splenin A activity with the anti-fibrinolytic effect of corticotrophin and cortisone. Splenin B reduced anti-fibrinolytic activity. In contrast to this Cliffton (1952) found that corticotrophin and cortisone in man caused a marked increase in spontaneous proteolytic activity of the serum, but no effect on the anti-proteolytic activity.

Stefanini and Murphy (1956) reported that platelets contained anti-fibrinolysin, either adsorbed or as part of their substance. This would be an opposing function to that of 5-hydroxytryptamine which is also contained in platelets and has been credited with initiating fibrinolysis (Kwaan, Lo and McFadzean, 1957b). Anti-fibrinolysins or anti-plasmins prepared from man, dog and the ox do not appear to show species specificity (Lewis and Ferguson, 1950a).

There is no great uniformity of comment about the anti-fibrinolytic activities in clinical states. For example, Biezenski (1960) reported a relatively constant anti-fibrinolysin level during pregnancy and that this did not differ from non-pregnant women. Naidoo, Hathorn and Gillman (1960) reported in the same volume that the anti-fibrinolysin titres often fell after delivery.

The list supplied by Sherry and colleagues of synthetic inhibitors of plasmin and plasminogen activation is as follows. Numerous organic substances, dyes and related compounds, heparin

and similar substances, basic amino-acids, their polymers and esters, quaternary amines, methyl amine and urea, lauryl amine, and E-aminocaproic acid. Also inorganic ions, particularly the heavy metals which inhibit or denature plasmin, and trypsin inhibitors such as pancreatic trypsin inhibitor, soya bean trypsin inhibitor and toxic phosphorus compounds. Barnett and Baron (1959) found an inhibitor of proteolysis in the supernatant fluid from some of their tissue cultures of human epidermoid carcinoma.

A CURRENT THEORY OF THE MECHANISM OF CLOT LYSIS.

An attractive current theory of the means by which fibrin clots are dissolved has been presented by Sherry, Fletcher and Alkjaersig (1959), based on their own work (Sawyer, Fletcher, Alkjaersig and Sherry, 1960). They claim to have shown that the rate of lysis of the clot is a function of the concentration of the activator of plasminogen surrounding the clot, and a function of the plasminogen concentration within the clot. If the surrounding medium is enriched with plasmin, the clot lyses no faster. They also showed that the lysis of clots *in vivo* was dependent on plasminogen activator concentration and independent of plasma proteolytic activity. Plasma inhibitors prevented much increase of circulating proteolytic activity, but were not active against the activator.

One of the significant points arising from this theory would be the devaluation of tests for plasma proteolytic activity. According to the theory, the ability of the body to dissolve its

clots and its exudates would be measured by the activator level and not by the proteolytic activity of the blood, the former being directly in proportion to the rate of lysis of the clot, however local and remote the clot might be, the proteolytic activity being a mere shadow of itself as a result of serum anti-plasmin activity. The theory is attractive, but has yet to be reconciled with the observation that activator and "proactivator" appear to be adsorbed on to fibrin together with plasminogen.

Fearnley (1953) allowed clots made from fresh plasma to remain in contact with the serum for one hour. He then removed them, washed them in saline and incubated them at 37°C in veronal saline buffer. All the clots underwent lysis. Clots similarly prepared from older plasma did not lyse, presumably because of loss of the labile activator. Since Fearnley's clots were immersed in veronal saline, it is hard to believe that lysis is proportional to the activator concentration in the medium surrounding the clot.

SOME TECHNIQUES USED FOR THE DEMONSTRATION OF FIBRINOLYSIS IN HUMAN BLOOD DESCRIBED BRIEFLY ACCORDING TO THE PRINCIPLES INVOLVED.

A. Techniques demonstrating the final common pathway of all fibrinolytic and anti-fibrinolytic influences in the specimen.

Some of the methods described probably alter the balance of pro-fibrinolysins and anti-fibrinolysins, but do so empirically.

1. Whole-blood clot lysis:

If fresh venous blood from a healthy person at rest is placed in a small sterile test tube kept at 37°C and observed at intervals, the blood will first clot and then will ordinarily remain as a firm clot for all of the first 48 hours. After 48 hours, observation is of questionable value. In the presence of sufficiently active fibrinolysis the clot will lyse within the first 24 hours and rarely, in considerably less time. The volume of blood used is commonly 1 ml. in each tube. The test will be discussed later.

2. Diluted whole-blood clot lysis:

Fearnley, Balmforth and Fearnley (1957) recommended observation of clots made from a dilution of whole blood. Blood obtained by venepuncture was kept at ice temperature and final dilutions made from this to 1:10 in phosphate buffer at pH 7.4, the mixture being clotted by thrombin. This was kept at ice temperature for a half to one hour, brought to body temperature

and then observed at intervals for lysis. Comment: Phosphate buffer was used because it was almost isotonic and did not lyse the red cells, although it had no superiority over veronal buffer when used for plasma dilutions (Fearnley, 1958). Lackner and Goosen (1959) appreciated how tied the laboratory worker becomes when practising a technique where observations may have to go on day and night for some time, and devised a photographic method, the camera taking half-hourly exposures indefinitely. The success of the photographs in this method depends on the release of red cells from the clot which is floating on the buffer. The red cells fall and settle at the bottom of the tubes.

3. Whole-plasma clot lysis:

If whole plasma clot is prepared similarly to whole blood clot, as in technique 1, it will be subject to similar lytic influences, but lysis is more easily observed. Moreover, observation is profitable for many more days than with whole blood clots. Plasma clot can be removed from its serum, resuspended in buffer and lysis still take place (Fearnley, 1953). Comment: Differences in lysis times from whole blood clots are often due to factors concerned in the preparation of the plasma clot, such as temperature, anti-coagulant, calcium, thrombin, pH and perhaps to the presence of red blood corpuscles themselves. Most of these points are discussed later. Partial lysis of clot is very difficult, usually impossible, to assess visually. Only where

lysis is rapid is there a time similarity with whole blood clots. The latter take longer to lyse if they are to do so at all.

4. Diluted plasma clot lysis:

Macfarlane in 1937 observed that clots made from plasma diluted 1:30 in saline lysed more rapidly and more inevitably than whole plasma clots. Biggs and Macfarlane (1953b) described a method using citrated plasma in buffered saline at dilutions of 1:16, 1:32, and 1:64. Thrombin was added and the clots observed for complete lysis at 24 hours or at other chosen intervals.

Fearnley and Lackner (1955) described a method involving serial dilution of ice cold plasma (see technique 6) from 100 per cent to 10 per cent, using 0.04 M. veronal buffer at pH 7.4 with 0.1 per cent calcium chloride. Clotting was spontaneous when the tubes were brought to 37°C and were observed hourly until lysis occurred. They found that the lysis time was a linear function of the plasma concentration provided the pH remained constant. In order to maintain a constant pH they added additional calcium veronal buffer to each tube some two hours after clotting had taken place. Comment: A method based on parts of the Fearnley and Lackner (1955) technique has been used here and points arising from the method will be discussed later.

5. Measurement of residual clot after incomplete lysis:

Bidwell (1953) described a method where a series of

identical plasma clots, in buffered saline, were incubated at body temperature. At regular intervals of about half an hour the clots were removed in turn, washed and treated with sodium hydroxide. The release of tyrosine-like substances was then assayed against a tyrosine standard using Folin - Ciocolteu's phenol reagent (British Drug Houses). A graph could then be drawn by joining the points representing amount of clot against the time of incubation. This gave some measure of the rate of lysis. Comment: The graphic presentation of results makes it easy to compare different experiments. Unfortunately the technique measures the amount of clot which is not lysed. This makes the technique least sensitive where most is required of it, namely the demonstration of slight degrees of lysis in whole plasma clots. This difficulty is only overcome by prior dilution of the plasma, but this adds an unknown factor to the interpretation.

6. Refrigeration techniques:

Fearnley, Revill and Tweed (1952), recognising the inactivation of fibrinolytic activity in shed blood at room temperature and at body temperature, developed a technique to avoid this. They set up their experiments at ice temperature, preparing different dilutions of plasma in buffer at pH 7.4. Only when the dilutions were prepared was the temperature raised to 37°C for clotting to take place. The clots were watched and the time necessary for complete lysis recorded. Comment: No anti-coagulant is necessary in these refrigeration techniques,

because blood which is chilled to ice temperature immediately it is withdrawn does not clot, at least for many hours. Thus no pro-coagulants such as calcium or thrombin are necessary. Refrigeration techniques demonstrate the weakness of many earlier methods where fibrinolysis was rarely demonstrated in the resting state of healthy people.

7. ^{131}I tagged fibrinogen and casein:

In vitro labelling allows one to follow the dissolution of human or bovine clot made with trace amounts of ^{131}I labelled fibrinogen. Lysis is measured by the radioactivity released into the surrounding solution in a given time. Comment: Methods using ^{131}I labelled fibrinogen and casein are rapid and apparently very reliable. Unlike the method of Bidwell (technique 5 above), the measurement represents the amount of clot lysed, instead of the amount of clot not lysed, and permits one to be independent of plasma dilution. Clement and McNicol (1959) described the preparation and use of ^{131}I tagged human fibrinogen and appeared to show in one of their figures that the increased rate of lysis after dilution was true and not merely apparent. The method is sufficiently sensitive to show lysis in unstressed normal people (Sawyer, Fletcher, Alkjaersig and Sherry, 1960). Heuson (1959) described the use of ^{131}I labelled casein.

8. Assay of lysis of whole blood clot by means of haemoglobin content of clot remaining:

Billimoria, Drysdale, James and Maclagan (1959) described

a method which ignored the direct effect of lysis on fibrin itself but measured the amount of haemoglobin (as red cells) trapped by the fibrin clot. Eighteen tubes of fresh whole blood in phosphate buffer, diluted approximately 1:20 and manipulated at ice temperature, were clotted by thrombin as aliquots of the blood were added to each tube. The temperature of the tubes was then raised to 37°C. Three tubes at a time were examined hourly for six hours. The clots were washed free of loose red blood corpuscles and then heated in sodium hydroxide. The resultant alkaline heamatin density was read on a spectrophotometer and the results plotted against time. The 50 per cent lysis times were noted. Comment: The accuracy of the method depends on the uniform enmeshing of red blood cells by each of the 18 clots. Results in triplicate must give confidence to what seems in theory to be an awkward part of the method, particularly in the presence of low fibrinogen levels. It is an advantage that all observations are objective, but it is uncertain as yet how the method compares in simplicity and reproducability with other methods.

9. Constant fibrinogen content in test and control:

Adelson and Roeder (1958) described five constituent experiments leading to a conclusion that the fibrinolytic activity in a sample bears an inverse relationship to the fibrinogen level.

The more the fibrinogen the less the demonstrable fibrinolysis. This justified their method. To aliquots of normal plasma, which they used as substrate, was added plasma defibrinogenated by heat, from the patient and from a normal person as control. After dilution between 20 and 30 times with saline, thrombin was added and the lysis of the resulting clot after 24 hours at 37°C was measured by tyrosine determination of the clots at the beginning and at the end of the incubation. Comment: It appears to an observer that the method has three main possible weaknesses. 1. It appears incorrect to be measuring fibrinolytic activity in plasma defibrinogenated by heat when some fibrinolytic agents are known to be heat labile. This point of criticism was anticipated by the authors and was defended by their demonstrating little difference when defibrinogenation was achieved by using thrombin; but plasminogen (and perhaps proactivator) may well have been adsorbed on to the fibrin clot which they had to remove. Thus both methods may be representing less than the true fibrinolytic content of the sample. 2. Plasma from a normal person, if it is to be used as a control, must itself be stringently controlled, because it may contain active fibrinolysins, such as can result from recent exercise and other recognised causes, all of which would make it unsuitable as a yardstick. 3. Saline is slightly inhibitory to fibrinolysis (Fearnley and Ferguson, 1958). Work which will be reported here is in disagreement with the claim that fibrinolysis bears an inverse relationship to the fibrinogen content.

10. Thromboelastograph recordings:

This is no more than an excellent graphic record of alterations in the physical state of blood and can be used to record either clotting or the lysis of formed clot, together with the time required for this to take place (Hartert, 1951: von Kaulla, 1957). Comment: The method requires expensive apparatus.

B. Techniques involving some analysis of the components of the fibrinolytic system.

11. Fibrin plate:

Permin (1947) described a method which has been used widely. Fibrinogen solution was poured thinly into a flat-bottomed glass dish and was clotted with thrombin. Measured drops of various solutions were then dropped on to the clot, these usually being derived from plasma. The lytic effect of such drops was followed at intervals, the degree of lysis being measured by the area of the clear space created in the semi-opaque fibrin. If such plates are heated to about 80°C adsorbed "proactivator" and plasminogen are destroyed, but the fibrin is apparently unchanged. This leaves the plates as indicators of protease activity in an added substance. Fibrin from human fibrinogen has adsorbed plasminogen and "proactivator", unless special precautions are taken. Fibrin from bovine fibrinogen has adsorbed plasminogen but not "proactivator".

Comment: Analysis of the fibrinolytic ingredients of test solutions has been made possible by using heated and unheated fibrin plates, and bovine and human fibrinogen. Many important observations have been made by the method, not least being the demonstration of tissue activator. In less experienced hands there were several technical difficulties in using the fibrin plate technique, the chief of which was the difficulty in obtaining a clearly demarcated and regular outline to the lysed areas such as would be suitable for accurate measurement. This applied particularly to plates made with bovine fibrinogen. Sherry, Lindemeyer, Fletcher and Alkjaersig (1959) have commented on this same point, finding incomplete zones of lysis when using whole plasma as the lysing agent. They found that euglobulin preparations were much more successful on the fibrin plates and left a clear-cut area to be measured. Bovine fibrinogen such as prepared commercially has rather too clear a clot to be ideal. Human fibrin prepared from fractionated plasma was more satisfactory in this respect.

12. Euglobulin fractionation:

Milstone (1941) added to 19 volumes of distilled water and 0.32 volumes of 1 per cent acetic acid, one volume of serum, and thus brought down the plasma euglobulin. When this was redissolved in saline he found that the resulting solution was more strongly lytic than the original plasma. Euglobulin fractionation has been used many times since,

sometimes with modification. Sherry, Lindemeyer, Fletcher and Alkjaersig (1959) regarded euglobulin fractionation as one of the more successful of the four methods they compared. Kowalski, Kopeč and Niewiarowski (1959) described the euglobulin method as valuable, particularly for comparative assay of fibrinolytic systems in large groups of patients. They elaborated the method together with a description of relevant biochemistry. The clots were observed every ten minutes until lysed. Comment: The rapidity of lysis makes close and regular observation possible until lysis is complete, and avoids the need for an automatic camera. The success of the method depends in large part on the separation of the fibrinolytic factors which come down in the precipitate and the inhibitors which remain in the supernatant.

13. Synthetic substrates:

Troll, Sherry and Wachman (1954) investigated the action of plasmin on synthetic substrates. They found that it attacked those synthetic amino-acid esters which have arginine or lysine as the amino-acid residue. This allows the use of synthetic substrates such as benzoyl arginine methyl ester or tosylarginine sulphonyl methyl ester, the use of which was described by Murray (1959). It was felt by Troll and his colleagues that the same enzyme centres are involved in the proteolytic, fibrinolytic and esterase activity of plasmin. Part of the evidence for this came from the way arginine and lysine esters act as competitive inhibitors to plasminogen activation. Kjeldgaard

and Ploug (1957) measured proteolytic activity by following the degradation of a heparin-protamine complex. Comment: There appears to be no differentiation in the synthetic substrate methods between fibrinolysin activity and trypsin activity. The method appears to suffer from the disadvantage which may be theoretical only, that a fibrin clot has to be removed from plasma before testing begins. Presumably variable amounts of plasminogen and perhaps proactivator of plasminogen are removed at the same time, adsorbed to the clot. What is left behind will then be measured.

Von Kaulla and Schultz (1958) compared different methods for assaying fibrinolysis, and favoured a combination of thromboelastography and a technique for determining euglobulin lysis time as against the use of synthetic substrates. Disappointing results with synthetic substrates were obtained by Schultz, Moorman, Matoush and Lincoln (1957), who measured fibrinolysis by lysine ethyl ester and tosyl arginine methyl ester assays. The method was sensitive enough to demonstrate variations between one individual and another, and variations in one individual from day to day and within the same day, but failed to display the increased fibrinolysis known to be present in certain sera. For this reason the method was condemned, but perhaps unjustifiably. It appears that their plasma was diluted 1:4 for the lysine ethyl ester and tosyl arginine methyl ester assays but 1:25 for the control assay by a more standard method of fibrin estimation. Moreover, the latter dilution was made with calcium chloride solution which is inhibitory to

fibrinolysis. For these two reasons it is felt that some reserve should be kept about the condemnation of the method made by the authors themselves.

14. Investigation into the activity of individual fibrinolysin precursors in the same specimen:

Phillips and Skrodelis (1958) used the lysis of casein as their indicator. They estimated "free pro-fibrinolysin" (plasminogen) in plasma by the amount of hydrolysis of casein after activation by streptokinase. "Total pro-fibrinolysin" was estimated similarly, using euglobulin in the place of whole plasma (the preparation of euglobulin removed the albumin inhibitor). "Active proteolysis" was measured by the hydrolysis of casein by plasma or euglobulin without activation by streptokinase. "Proenzyme content" is calculated by subtracting active proteolysis from streptokinase activated lysis. Inhibitor activity is calculated by subtracting the streptokinase induced lysis of plasma from that of euglobulin. Comment: This technique applies a more fundamental approach to the study of fibrinolysis and is an attempt to analyse which component of the fibrinolysis system is responsible for the waxing and waning of fibrinolysis on different occasions. Such an approach is most desirable, although some uncertainty is felt whether the methods in use are exact enough for this purpose.

15. ^{131}I tagged fibrinogen and streptokinase:

Although described in technique 7, this method is also well suited for the critical analysis of individual components

of the fibrinolysis system (Sawyer, Fletcher, Alkjaersig and Sherry, 1960). Kline and Fishman (1957) labelled streptokinase with ^{131}I, with apparent success.

16. Anti-fibrinolysis (anti-plasmin) assay:

Sherry, Fletcher and Alkjaersig (1959) felt that anti-plasmin assays were not worth while unless there was a source of potent plasmin, uncontaminated by activator. Their technique supplied plasmin of the required potency and purity by auto-activation of plasminogen, glycerol being used as a stabilizer. This approach to the problem of anti-plasmin assay seems the only correct one for analytical work demanding results in terms of an absolute, if arbitrary, unit.

Satisfactory results have been obtained by others using methods which are open to some criticism on theoretical grounds, but which give in practice reproducible results, suitable for comparing the anti-fibrinolytic activity of different patients. Biezenski (1960) prepared 12 serial dilutions of potent plasmin which he prepared by streptokinase activation of plasminogen derived from human fraction III, by Cliffton and Cannamela's modification (Cliffton and Cannamela, 1953) of Kline's method. To each dilution of plasmin he added an equal volume of diluted serum from the patient and after ten minutes incubation he added human fibrinogen and thrombin. The anti-fibrinolytic titre was expressed as the lowest dilution of the plasmin which was inhibited by the constant amount of serum

added. The control with saline in the place of serum resulted in the "absence of clot" in all the tubes. This is presumed to mean clot-lysis in all tubes.

PHYSIOLOGICAL, PATHOLOGICAL AND EXPERIMENTAL CONDITIONS ASSOCIATED WITH AN INCREASE OF BLOOD FIBRINOLYSIS.

Physiological:

The part played by adrenalin in augmenting fibrinolysis has already been described and is the best understood mechanism involved in physiological increases of fibrinolysis. In any example of increased fibrinolysis it is impossible to tell how much of the increase is due to adrenalin and how much may be due to other factors.

Pathological:

Largely owing to the work of Tagnon, Whitmore and Shulman (1952), the association of carcinoma of the prostate with abnormal fibrinolysis is widely accepted. The incidence of this combination is as yet undetermined with certainty, but was quoted by Tagnon, Whitmore, Schulman and Kravitz (1953) as 12 per cent of their cases of metastatic carcinoma of the prostate.

Cirrhosis of the liver has been associated with abnormal fibrinolysis for a long time (Goodpasture, 1914: Kwaan, McFadzean and Cook, 1956). An association with abnormal fibrinolysis has been described for carcinoma in various sites such as stomach, breast and disseminated carcinoma of the bladder. Fibrinolysis in multiple myeloma has been described and has been attributed to cryoglobulin of the Waldenström type and to diminution of plasma albumin with its associated anti-proteolytic activity (Sirridge, Bowman and Garber, 1958). Systemic lupus erythematosus has been described in association with fibrinolysis. Burns and whole body irradiation have been associated with fibrinolysis, as have ulcerative colitis, rheumatism, nephritis, lead poisoning, urticaria, Raynaud's phenomenon, iritis, serum sickness, pregnancy toxaemia and obstetrical accidents (Ratnoff, 1949) and polycythaemia vera (Bjørkman, Laurell and Nilsson, 1956). Pulmonary and pancreatic disease have both been described with an increase of fibrinolysis, associated by Murray (1959) with tissue destruction. Acute leukaemia has not infrequently been associated with abnormal fibrinolysis (Pisciotta and Schulz 1955: Cooperberg and Neiman, 1955: Hillestad, 1957: van Creveld and Mochtar, 1960). Increased fibrinolysis in the girl suffering from acute leukaemia described by van Creveld and Mochtar was shown to have its origin in her platelets rather than in the plasma. This list is by no means exhaustive but

is sufficient to show the absence of obvious specific common factors between the various disorders which have been described in association with increased fibrinolysis.

Study of published reports of fibrinolysis in disease shows that trauma has often been associated with the demonstration of abnormal fibrinolysis and it is a point for discussion how great a part is played by the underlying disease process in the cases described and how much by the trauma itself. Abnormal fibrinolysis was found during hepatic lobectomy for local malignancy in five out of six patients when this was looked for, but clinically there was only surgical oozing (Zucker, Siegel, Cliffton, Bellville, Howland and Grossi, 1957): during splenectomy (Kwaan and McFadzean, 1956) where in one case the plasma clot lysed in four minutes: during surgery of benign adenoma of the prostate (Rasmussen and Ladehoff, 1959): in four patients suffering from shock (Coon and Hodgson, 1952): and after cardiac arrest following Caesarean section (Heuson, Peers and Tagnon, 1958). Three examples of abnormal bleeding attributed to fibrinolysis were described by Firkin, Reed and Blackburn (1957) in association with operations for pilonidal sinus, fissure-in-ano and a dental operation.

Experimental:

Local effect: Kwaan, Lo and McFadzean (1957b) injected 5-hydroxytryptamine paravenously and demonstrated a consequent increase in fibrinolysis within the same vein and

in the opposite limb. They suggested that the action of the 5-hydroxytryptamine (like that of adrenalin and ischaemia) was mediated by a cholinergic mechanism, and implied that an important function of platelets was to initiate fibrinolysis by virtue of the 5-hydroxytryptamine contained in them. Procaine injections in the vicinity of an artery or vein led to the development of fibrinolysis in the blood contained in the vessel (Kwaan, Lo and McFadzean, 1958a).

General effect: Peptone shock was used by Nolf in 1905 as an experimental technique which stimulated fibrinolysis in the blood stream. Nicotinic acid when injected (but not nicotinamide) was shown by Weiner, de Crinis, Redisch and Steele (1959) to increase fibrinolysis, as did a small dose of heparin in the presence of albumin (von Kaulla and McDonald, 1958: Lackner and Merskey, 1959). Aspergillin O was purified with a view to therapeutic use as a lytic provocateur, and was tried on a limited scale but does not appear to have been followed up (Stefanini, Adamis, Soardi, Horace, Marin and Mele, 1959). Insulin injections produced a biphasic response, the increase in fibrinolysis being on the rebound and probably attributable to adrenalin secretion in response to hypoglycaemia (Fearnley, Vincent and Chakrabarti, 1959). After unsuccessful trials of several other preparations, Fearnley, Chakrabarti and Vincent (1960)

obtained a sustained increase of fibrinolysis in six out of eight patients after the administration of sulphonylurea.

One of the points relating to technique in measurement of fibrinolysis is the citrate effect which increases the lytic activity of the isolated euglobulin fraction of human plasma. This may do so by increasing activator activity (Buckell, 1958).

Thrombolytic activity: Streptokinase itself was used successfully as an injection, in dogs and in man, by Sherry and Alkjaersig (1957). Streptokinase-activated plasmin has also been used (Grossi, Cliffton and Cannamela, 1954: Back, Ambrus, Simpson and Shulman, 1958). Ruegsegger, Nydick, Hutter, Freiman, Bang, Cliffton and La Due (1959), using plasmin, treated experimentally induced coronary thrombosis in dogs with apparent success. Chymotrypsin was used for therapeutic lysis of experimentally induced arterial thrombi in dogs (Keirle, Glueck, Neely and Altemeier, 1960).

In man similar results appear to have been obtained (Cliffton, 1957: Ambrus, Ambrus, Back, Sokal and Collins, 1957). Ten patients with thirteen episodes of thrombophlebitis were treated with various forms of fibrinolysin (often activated with streptokinase) and there was dramatic recovery, although no immunity to further thrombosis (Sokal, Ambrus and Ambrus, 1958). Moser (1958) used streptokinase activated euglobulin in 52 patients with various forms of thromboembolic disease. This gave rise to "cautious optimism".

It was therefore followed by a controlled study of the effect on acute deep thrombophlebitis of plasmin therapy, given usually by a three-hour infusion. This régime appeared to give benefit (Moser, Sulavik and Hajjar, 1960). Sussman and Fitch (1958) treated three patients suffering from recent hemiplegia with a slow infusion of fibrinolysin (plasmin) after locating the site of occlusion by arteriography. One patient showed partial clearing of the internal carotid lumen after treatment. Another patient's arteriographs showed no filling of the middle cerebral group of arteries before treatment but good filling eight days after treatment was begun. The authors failed, of course, to prove that it was their therapy which led to the improvement.

PHYSIOLOGICAL, PATHOLOGICAL AND EXPERIMENTAL CONDITIONS ASSOCIATED WITH AN INHIBITION OF FIBRINOLYSIS.

Predominantly physiological:

From the early months of pregnancy onwards and during labour, inhibition of fibrinolysis has been demonstrated, although fibrinolysis returned to normal within the first 24 hours after delivery (Biezenski and Moore, 1958: Gillman,

Naidoo and Hathorn, 1959). The inhibition was attributed to a reduction in lytic enzyme rather than to increased anti-fibrinolytic activity (Biczenski, 1960). Alimentary lipaemia appears to depress fibrinolysis (Greig, 1956: Buckell and Elliott, 1959b: Billimoria, Drysdale, James and Maclagan, 1959), possibly by means of the increase of beta-lipoprotein and chylomicra which are inhibitory (Greig and Runde, 1957). Mitchell and Briers (1959) found no depression of fibrinolysis as a result of suspensions of cholesterol esters and emulsions of neutral fat prepared in the laboratory, but found that cholesterol oleate suspensions were inhibitory. Nitzberg, Peyman, Goldstein and Proger (1959) found an association between the fibrinolysis time and triglyceride levels and serum turbidity.

Predominantly pathological:

Kwaan, Lo and McFadzean (1959) demonstrated inhibition of fibrinolysis in primary carcinoma of the liver as well as in some other malignant tumours. They felt able to suggest that the test might be used as an indicator of the onset of carcinoma of the liver in a patient already suffering from cirrhosis. In such a patient fibrinolysis would remain responsive to adrenalin and ischaemia until the onset of malignancy. The inhibitor responsible could be extracted in saline from the tumour itself and was active against activator of plasminogen and was not an anti-plasmin. Hume (1958) showed inhibition of fibrinolysis in venous blood for

eight days after myocardial infarction. Lackner and Merskey (1959) found that there was fluctuation of lysis times after myocardial infarction, although on the whole, lysis times were longer at first. Nestel (1959) demonstrated depression of fibrinolysis in a group of 30 men suffering from intermittent claudication. Fearnley, Vincent and Chakrabarti (1959) found that fibrinolytic activity in diabetes mellitus was reduced initially by insulin injections and suggested that this, together with the rebound fibrinolytic effect when adrenalin is secreted in response to hypoglycaemia, might be related to carbohydrate metabolism. A deficiency in lung fibrinolysis has been demonstrated in hyaline membrane disease by Lieberman (1959) and Lieberman and Kellogg (1960), as also after radiotherapy to the lungs (Fleming, Szekacs, Hartney and King, 1960).

Predominantly experimental:

Exercised ischaemic muscles appear to play an inhibiting role on fibrinolysis (Kwaan, Lo and McFadzean, 1958a). The clotting process appears to mask lysis which would otherwise be demonstrable in thrombin-clotted specimens (Fearnley and Ferguson, 1958). Red cell stroma is fibrinolytic, according to Permin (1947). Calcium has been used relatively freely in fibrinolytic systems set up in the laboratory both before and after the demonstration by Fearnley and Tweed (1953) that calcium partially inhibits fibrinolysis. Calcium as the only

clotting agent leads to very considerable inhibition of blood lysis (Fearnley and Ferguson, 1958). An inhibitory effect of calcium has been confirmed here. According to Medart (1958) calcium does not inhibit the activity of plasmin, but stabilises the substrate fibrin. Even saline has been incriminated as an inhibitor of fibrinolysis (Fearnley and Ferguson, 1958). Large doses of heparin have inhibited fibrinolysis (von Kaulla and McDonald, 1958). Beer was shown by Fearnley, Ferguson, Chakrabarti and Vincent (1960) to be inhibitory to fibrinolysis. In this respect at least, beer equalled white wine. The first observations on the therapeutic use of the soya bean trypsin inhibitor were made by Heuson, Peers and Tagnon (1958) who injected the substance intravenously into a patient who was bleeding profusely on the occasion of cardiac arrest following Caesarean section, with resultant rapid disappearance of fibrinolysis in her blood. The patient died later without further bleeding. The Lima bean has also been used successfully in laboratory experiment and appears to act in a similar way to the soya bean trypsin inhibitor (Lewis and Ferguson, 1953). Corticotrophin has been shown to lessen or stop the lysis of experimental ear vein thrombosis in rabbits (Kwaan and McFadzean, 1956a). Corticotrophin, cortisone and prednisone have been reported to inhibit the fibrinolysis of cirrhosis (Kwaan and McFadzean, 1956b) and

that produced by 5-hydroxytryptamine but not that induced by adrenalin (Kwaan, Lo and McFadzean, 1958b). These writers made the position even more confusing by claiming that cholesterol-induced inhibition (of fibrinolysis) inhibited both the action of 5-hydroxytryptamine and of adrenalin and was thus distinct from the corticotrophin action. Stefanini and Gendel (1953) reported favourably on the use of cortisone in "fibrinolytic purpura", and reported one such case associated with prostatic carcinoma. Epsilon-aminocaproic acid was used for the first time in man by Nilsson, Sjoerdsma and Waldenström (1960). They claimed significant depression of fibrinolysis for at least seven hours after oral or intravenous therapy. An apparently isolated report of the success of toluidine blue in inhibiting fibrinolysis arising in patients with carcinoma of the prostate came from Lombardo (1959).

PHYSIOLOGICAL FLUCTUATION IN FIBRINOLYSIS

There is a diurnal variation of fibrinolytic activity. Fearnley, Balmforth and Fearnley (1957) recorded the lysis times

of blood taken from fifteen day nurses at 4 a.m. (twelve of them being asleep) and from the same girls when they were up and about at 4 p.m. They also recorded the blood lysis time in fifteen night nurses on duty at 4 a.m. and in the same girls at 4 p.m. when twelve of them were asleep. The results from the day and night nurses were very similar. Less fibrinolysis appeared to take place at 4 a.m. than at 4 p.m. The physical activity which is involved by night-duty did not upset this rhythm in the majority, only one night nurse lysing faster at night. Buckell and Elliott (1959a) confirmed these findings in men, using Bidwell's (1953) technique and the euglobulin lysis time as described by Buckell (1958). They found that the variation was greater in the younger subjects. Billimoria and colleagues (1959) estimated the haemoglobin remaining in diluted whole-blood clots after exposure to lysins. They agreed about there being a diurnal variation in fibrinolysis but found it to be significant only when the subjects were not in bed.

THE SIGNIFICANCE OF THE LOCATION OF FIBRINOLYTIC FACTORS

It may appear that the site of production, or of residence, of fibrinolytic factors is of as little apparent importance as the site of production of clotting factors. This is by no means so. Over many years a massive literature has accumulated around the subject of fibrinolysis, concerning its increase, and its lessening, and its component factors.

There is hardly a physiological or pathological state in which fibrinolysis has not been assayed. Yet one can still question the basic significance of fibrinolysis. The part played by fibrinolysis in physiology is mere conjecture and the part it plays in disease is quite uncertain. Against this background the site of production of fibrinolytic factors assumes greater significance if only because a study of it permits fresh conjecture on the more fundamental problems of fibrinolysis.

The relatively insoluble tissue activator has been demonstrated in most tissues, although Albrechtsen (1957) found liver tissue to be almost inactive. Most tissues are therefore a ready source of local fibrinolytic activity which is possibly independent of a generalised whole-body response. Tissue fibrinolysis may play a part in local response to injury by restricting the spread of fibrinous damage with its sequel of fibrosis. It is possible that the emergence of healthy alveolar walls from a lung lately swamped by lobar pneumonia, can be attributed in part to the defensive action of the tissue activator which is known to be present in the lung. In contrast to this, tissue activator has been shown to be congenitally absent in lungs affected with hyaline membrane formation. Lieberman (1959) and Lieberman and Kellogg (1960) first showed this in the new born, and Fleming, Szakacs, Hartney and King (1960) showed the same absence of tissue activator in the lungs of two men who died shortly after radiation to the chest and

who had the typical hyaline membrane formation. Spill-over of tissue activator is a potential source of plasma activator, although some physico-chemical change would be required because plasma activator is so much more soluble.

The activator and proactivator content of tears, milk, saliva, urine and prostatic secretion is easily rationalised as a means of keeping narrow ducts clear and free from clot.

The fibrinolytic activity of whole blood is assayed most often, but can be rationalised least well. Certain observations and experiments give a lead. There appears to be a difference between the fibrinolytic activity of arterial and venous blood. Chaplin (1954) found fibrinolysis to be three times greater in the umbilical cord artery than in the vein. Fearnley and Ferguson (1957) found that the lytic activity in arterial blood was less than in venous blood using arm vessels. They made the observations that venous blood lysis time is no measure of arterial lysis time and that the lytic agent may arise in the periphery. Mole (1948) had already suggested that "the fibrinolysin" was produced by the endothelial lining of the vascular channels, particularly peripheral ones, and body cavities. Kwaan, Lo and McFadzean (1957a: 1957b) injected adrenalin, acetylcholine or 5-hydroxytryptamine intravenously, or paravenously, into limbs with restricted

outflow of blood and demonstrated an increase of lysis in the segment concerned. Particularly interesting was the simultaneous increase of lysis in the vein of the opposite limb, apparently mediated by a neurological reflex. Atropine inhibited both the local and the reflex increase. Arteries and capillaries seem capable also of a similar production of fibrinolytic activity. Their deductions were that fibrinolytic activity may be attributable to ischaemia of vessel walls, possibly secondary to constriction of the vasa vasorum. This ischaemia causes stimulation of some cholinergic mechanism, resulting in release of activator into the circulation. The main source for this agent thus may be the vessel walls. This allows one to postulate that the function of the fibrinolysin found in the circulating blood is that of maintaining a free channel for the blood.

There seems to be no particular location known for inhibitory factors. Those which have been recognised have, for the most part, been demonstrated in the blood stream. There their function may be to remove what is left over from a local excess somewhere in the body. Alternatively, their most important action may be to block the activity of circulating lysins so that the latter are effective only when adsorbed on to fibrin. This latter view is widely held.

CONGENITAL ABERRATIONS OF THE FIBRINOLYTIC SYSTEM.

Proof has been lacking that fibrinolysis is important to the healthy body. In other branches of medicine it has sometimes required a congenital deficiency to draw attention to the physiological function of a substance or a body secretion. In the study of fibrinolysis in the past, there has been no demonstrated example of a congenital deficiency of a fibrinolytic factor with an associated dysfunction or pathology inevitably associated with it, and so acting as a marker of the abnormality. It is therefore of particular interest to study the reports of Lieberman (1959) and Lieberman and Kellogg (1960) to which reference has already been made. It would appear that fatal hyaline membrane disease of the newborn is, in their early experience, inevitably associated with a deficiency of tissue activator in the lung. They showed that tissue activator normally appears as early as the third month of gestation. There was suggestive evidence of tissue activator deficiency in organs other than the lung, notably the pancreas. There was an unproved impression that the condition of hyaline membrane disease of the newborn is familial, the mother of such babies tending to have multiple neonatal deaths suggestive of hyaline membrane disease. If the connection between these factors is ever proved, it would be the first example of a hereditary

failure of fibrinolysis, and its further development would help greatly in displaying at least part of the physiological role of tissue activator.

CHAPTER TWO

METHODS, MATERIALS AND NATURE OF RESULTS

1. PLASMA FIBRINOLYSIS ASSAY

Introduction

The method of assaying fibrinolysis used throughout this work is a modification of that described by Fearnley and Lackner (1955) unless specifically stated to the contrary. The choice of this method was determined more by a chance of timing than as a result of exhaustive study of different methods. Until 1956 we had measured fibrinolysis by the method of Bidwell as quoted by Biggs and Macfarlane (1953b). This has been described briefly in chapter one, technique 5. The newly published method of Fearnley and Lackner had much to commend it and justified trial. It observed the lysis of whole plasma as well as a series of dilutions of plasma down to 10 per cent. All manipulations were performed at ice temperature, which preserved the labile factor involved in fibrinolysis and rendered anticoagulants and procoagulants unnecessary. Specimens were then brought to body temperature for clotting to take place and were observed at intervals for clot lysis. It was hard to imagine a more untampered *in vitro* technique for the study of fibrinolysis and this seemed to commend the method.

About this time, a patient who had presented dramatically with hypofibrinogenaemia during minor surgery to the prostate was reinvestigated for plasma fibrinolysis using the chilled dilution method. Another patient showed

unusually powerful fibrinolysis, using the same method. Such was the interest aroused by these patients that it was felt undesirable to change the method of assaying fibrinolysis until the significance of the findings on them was made clear. To some extent this thesis has developed in an endeavour to put into perspective the findings in these two patients. Clearly it was impossible to change methods in the middle, without losing much of the information already gathered, even though a change was seen eventually to be desirable because of difficulty in presenting results. Nevertheless, modifications of the method were frequently run in parallel when there was enough plasma. Results from these experiments in parallel have not been presented as a separate study because they were changed from time to time and in no case, except where they duplicated the method exactly, did they appear to be giving as helpful information as was already being received. It was not felt reasonable to break away entirely from the dilution method so long as the aims of the investigation remained unchanged.

The technique described below is applicable to all cases in the main series and in the control series unless otherwise stated.

Equipment used:

Container with broken ice.

Broad elastic bands to support test tubes suspended in the ice.

Sterile

- 3 test tubes, 1½ by 10 cm., round bottomed, toughened glass, siliconed.
- 1 siliconed 30 ml. all glass eccentric nozzle syringe with a 1½" needle of 19 standard wire guage external diameter.
- 11 test tubes 12 by 75 mm. (3" by ½"), in rack.
- 2 graduated 1 ml. pipettes.
- 1 Pasteur pipette with rubber teat.

Centrifuge - MSE Multex, with 50 ml. buckets.

Incubator at 37°C.

Pye Universal pH meter.

Buffer:

Veronal buffer was prepared at pH 7.4, using a mixture of 58.1 ml. of 0.1 M. barbitone and 41.9 ml. of 0.1 M. HCl. In such assays as have been so marked the same buffer was used with the addition of 0.1 per cent calcium chloride.

The buffer was put into universal containers in appropriate amounts for each experiment. These were then autoclaved in one batch, so that sterile buffer was always available in small amounts. The pH was rechecked on an aliquot after sterilization.

Glassware:

Washing of glassware included preliminary brushing and rinsing, boiling in "steroxyl" (Laporte Chemicals

Ltd.), further brushing under running water and then triple rinsing, followed by boiling in distilled water and oven drying. Tubes were plugged with cotton wool before dry air sterilization. Pipettes were cleaned in chromic acid and rinsed many times in tap water and distilled water before drying by passage of acetone. Pasteur pipettes were cleaned similarly and were plugged with cotton wool. Siliconing of syringes and large tubes was repeated only at intervals. Syringes were washed and sterilized by dry heat inside an individual container. It was found convenient to wrap up all the pipettes required for one experiment in a large brown paper envelope and they were sterilized in this. None of the glassware was used for any other purpose.

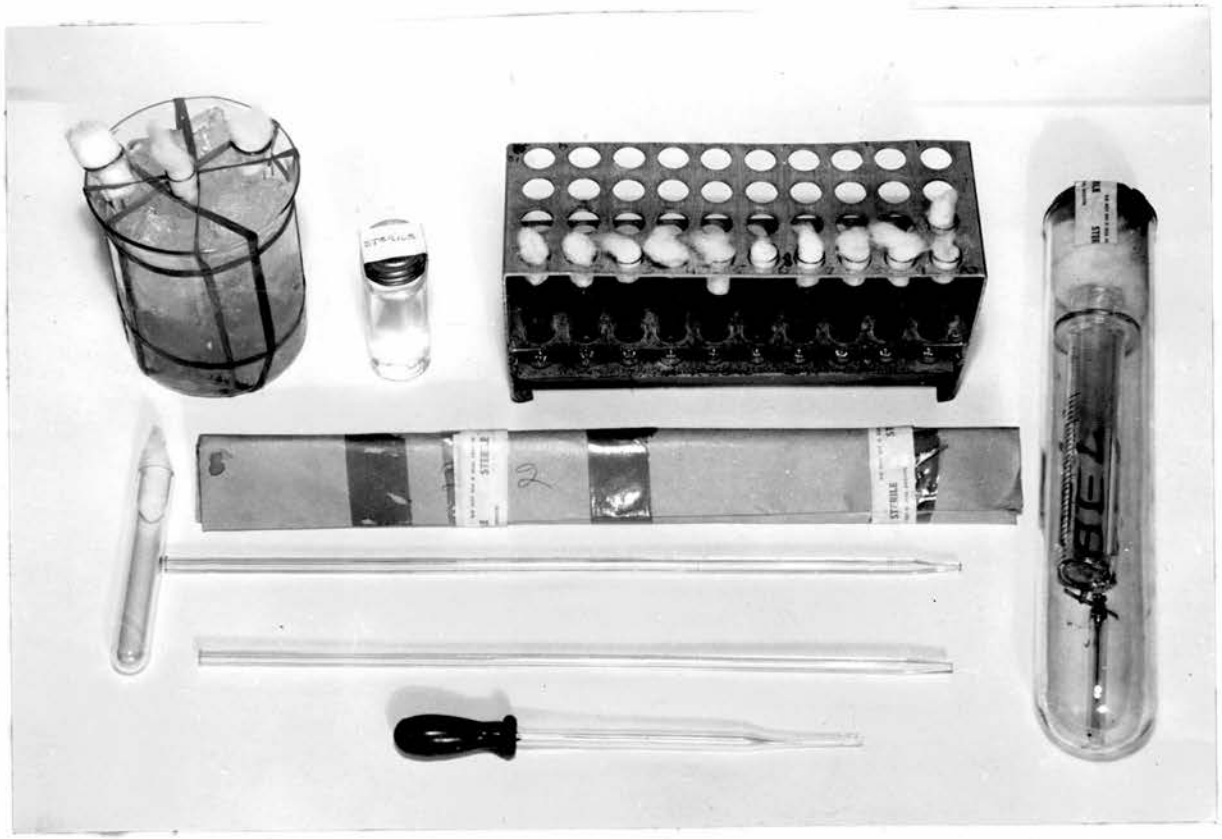


FIG. 1: The equipment required for one experiment using the chilled dilution technique. The pipettes are shown without their wrappings. An additional parcel of pipettes has been shown in order to demonstrate the form in which the pipettes were sterilized.

Technique:

All venepunctures were made by the same person, with the exception of about ten instances, and blood was removed, with few exceptions, at 10 a.m. or within one hour on either side. All patients had eaten a hospital breakfast and were still in bed. A sphygmomanometer cuff round the arm was blown up in all cases to 100 mm. Hg. immediately before insertion of the needle. Samples were acceptable only if the venepuncture was clean. 32 ml. of blood were withdrawn into a syringe and then the blood was allowed to run gently into two 15 ml. test tubes suspended in broken ice until each tube was filled to near the level of the cotton wool bung, which usually allowed 12 ml. in each tube. One ml of the blood remaining in the syringe was run into a small test tube for subsequent observation of lysis. The remainder of the blood was added to potassium oxalate (approximately 2 mg. per ml. of blood) for fibrinogen estimation. This arrangement permitted considerable uniformity of timing, specimens from other hospitals being manipulated after the same interval as from the wards of the same hospital. No anticoagulant was used at this stage, or subsequently, in the fibrinolysis assay.

The test tubes containing the blood were transferred from the beaker of ice to 50 ml. centrifuge buckets and were packed around with more broken ice and ice water. These were balanced and centrifuged at 2,000 revolutions a minute for ten minutes.

While the blood was being centrifuged, buffer was pipetted using a sterile technique, into a row of ten sterile test tubes in a rack, each tube with its cotton wool plug. The first tube was left empty: 0.1 ml. of buffer was put into the second tube: 0.2 ml. into the third tube: 0.3 ml. into the fourth tube and so on, until 0.9 ml. into the tenth tube. Usually a second row was prepared for duplication or further experiment.

The centrifuged plasma, still ice cold, was removed from the packed red cells by a Pasteur pipette and transferred into another 15 ml. sterile test tube suspended in the container of ice. From this the plasma was pipetted, using one of the graduated pipettes, into the ten test tubes in the rack, such that each tube ultimately contained 1 ml. of liquid. This involved transferring 1 ml. of whole plasma into tube 1: 0.9 ml. into tube 2: 0.8 ml. into tube 3 and so on, until 0.1 ml. into tube 10. Immediately after each quantity of plasma was added, the appropriate tube was shaken briefly to mix the buffer intimately with the plasma. The rack then contained ten tubes of plasma in serial dilution from 100 per cent to 10 per cent, and this, together with the tube containing 1 ml. of whole blood, was placed in the incubator. No procoagulant such as thrombin was added. It was usual for the rack to be placed in the incubator half an hour after the venepuncture. Clotting never occurred before incubation began.

The tubes were observed after approximately half an hour to make sure that a clot had formed in each tube.

Thereafter the tubes were examined at intervals until each clot had lysed. The technique found to be most satisfactory for examination of the clots was to tap each tube once or twice, observing the tube meantime against fairly bright artificial light. This sometimes betrayed the presence of a ghost clot which vanished on such gentle agitation. It was felt with experience that unnecessarily vigorous tapping would not usually disrupt a normal clot and that error would more likely stem from failure to tap, rather than from over-shaking. The end point was regarded as complete disintegration of clot. Not infrequently there still remained at the bottom of the tube a small amorphous sediment which did not disappear even after further incubation.

At first a great many of the tubes were cultured after lysis had taken place to see whether infection could have played any part in the lysis. Cultures were invariably sterile. It was decided eventually that the technique had been shown to be sound, so far as contamination was concerned, and that further culturing was unnecessary. It was considered that a turbidity would certainly develop in plasma incubated for several days at blood temperature if the specimen had been contaminated. This never happened, and it seems safe to claim that the lysis of the plasma clots was not influenced by any bacterial contamination.

After the setting up of an experiment it would have been ideal to observe each tube every hour until lysis took

place. Lysis, however, proved to be utterly unpredictable in the early stages for any one particular patient. It was possible to observe the tubes assiduously for almost 12 hours and then to leave the laboratory finally for the night, fully satisfied that nothing was happening. By morning, however, several tubes, and in some cases all tubes, had lysed. It was therefore only by desire that tubes were observed hourly or two hourly until lysis took place. In practice overnight gaps existed. It was partly for this reason that such care was taken to make sure that experiments were set up at the same time of day, and also not later in the week than Wednesday, in order that the observations due on Saturday and Sunday could be made less frequently without disturbing comparative values. Almost all the control series fell under this more strict regime. The patients who had carcinoma of the prostate had to be accepted for examination as opportunity presented and the day of the week was difficult to control. It was usually possible to arrange to take the specimen of blood at about 10 a.m.

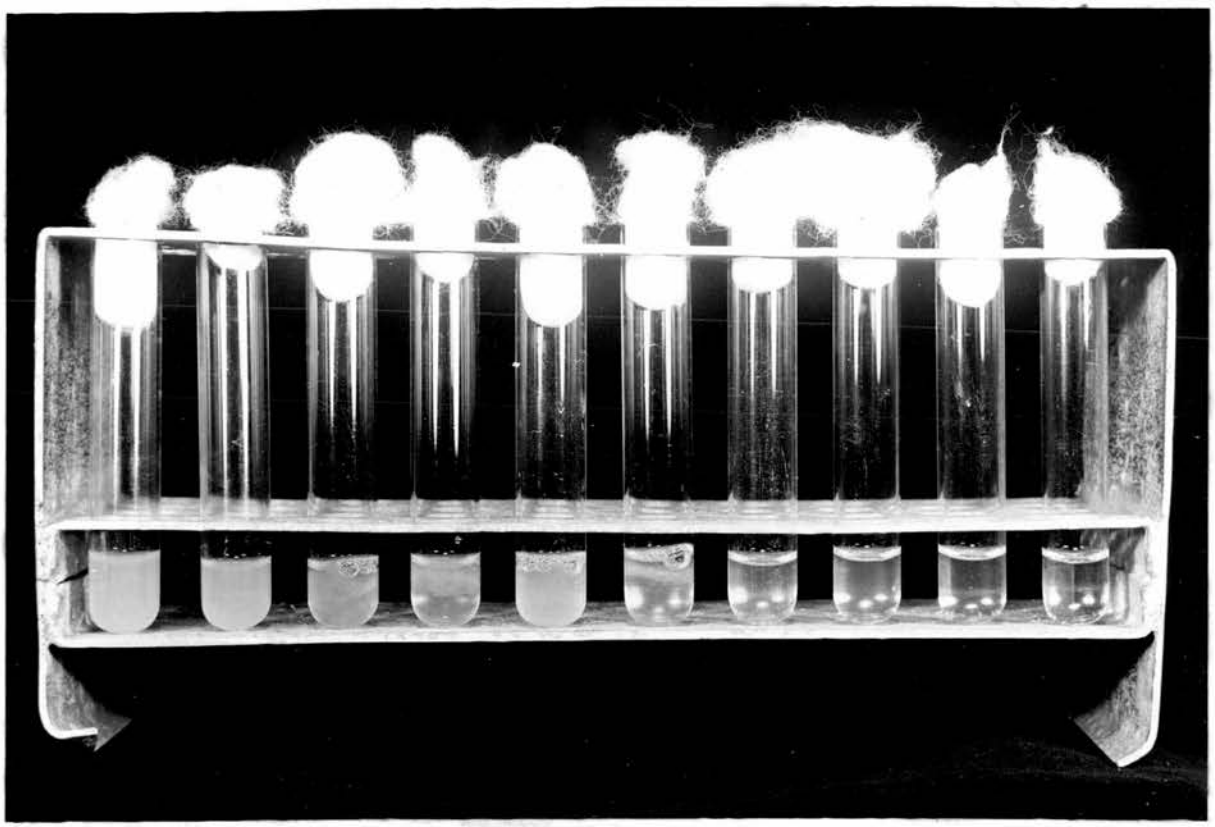


FIG. 2: An experiment in progress. From right to left the tubes are in sequence, rising from 10 per cent plasma on the extreme right to whole plasma on the extreme left. The 10, 20, 30 and 40 per cent clots have lysed completely. The 50, 60, 70 and 80 per cent clots are partially lysed (this is not apparent in the photograph for the 60 per cent clot). The 90 and 100 per cent clots show no evidence yet of lysis.

The nature of the results obtained:

This must inevitably involve some discussion of the method itself, but it is necessary to do so at this stage in order to explain the type of results obtained and the steps taken to render them suitable for comparative purposes. The raw material for interpretation, obtained at the end of each experiment, was a series of ten figures. These represented the lysis time in hours of each of the ten plasma clots which were made, in the first place, from plasma in serial dilution from 100 per cent to 10 per cent. The dilute plasma clots usually lysed first, lysis proceeding in sequence towards the whole plasma clot.

When Fearnley and Lackner (1955) used their technique, which was almost identical apart from a larger clot and the use of calcium veronal buffer, they found that they were able to plot their results as a sigmoid curve but not as a straight line. They proceeded to show that this was due to a rise in pH, most apparent in the concentrated plasma clots. This change in pH could be obviated by layering excess of buffer on top of each clot some two hours after incubation. By such means the lysis times in the ten tubes formed a straight line when plotted against the dilution. The general slopes of the sigmoid and the straight lines were similar. Fearnley was using buffer containing calcium, but in our hands the layering on of buffer containing calcium at pH 7.4 did not always give a straight line. Indeed, the clots sometimes

seemed loath to lyse at all and the parallel experiments, set up as described above, seemed more satisfactory. For this reason Fearnley's layering method was abandoned, although it is felt in retrospect that this may have been done for the wrong reason. A change of buffer, particularly to one without calcium, might have been more successful. It would appear, however, that a straight line relationship between the lysis time and the concentration cannot be expected by the method used here. Indeed, it was virtually never found.

Even if one accepted the apparent impossibility of plotting the ten figures from each experiment as a straight line, other alternative graphic presentations might have been possible. Certain important differences between patients were, however, obvious. It was seen that the general pattern of lysis in a minority of experiments was totally unlike that in the majority, and in the experiments presented by Fearnley and Lackner. To take one example, the whole plasma clot occasionally lysed first. It lysed before the 10 per cent plasma clot in six out of 101 consecutive assays. This prevented easy comparison of different experiments.

In addition to variations in the overall pattern of lysis between different patients there was no constancy about the smooth flow of lysis times from each tube to its neighbour within the same experiment. The explanation for this does not seem to reside in the pH, even although the pH varied with the different concentrations of plasma. The lysed clots from 13 experiments were examined for pH, and a smooth flow of change

was always observed from one tube to its neighbour. The mean rise of pH was from 7.4 in the 10 per cent tube to 9.1 in the 100 per cent tube.

Obvious errors of technique are not likely to be responsible for the irregularity because the same person, practising care and using the same technique, performed almost all the 10,000 pipettings of plasma required. The cause of the tube to tube inconsistency remained unanswered and would appear to be a weakness in the method. It is felt that very minor points may have been responsible.

With a view to discovering the best method of presenting the results from all experiments, the available figures were studied. It seemed appropriate from a statistical approach to present the results of each experiment as a single figure using the 50 per cent plasma clot lysis time, thus disregarding the remaining dilutions. Here an attempt will be made to show by diagram that this decision was reasonable and that the 50 per cent clot lysis time is an optimum point on the main trend of the lysis times in all experiments.

Attention will be restricted at this stage to all of 101 assays performed during an arbitrary period of nine months during the period of greatest activity in the laboratory when the technique was well established and was uniformly practised. The cases included 11 of carcinoma of prostate and 90 from the control series of both malignant and non-malignant conditions. The technique followed was identical

in all cases and the buffer never contained calcium. The ten lysis times from each experiment were plotted against the initial plasma concentrations and the general shapes of the resulting graphs were studied. Three figures have been prepared to show three extremes of shape obtained. Although drawn from actual experiments, these figures should be accepted for their general outline and not for the significance of individual points on them.

Most of the assays conformed in general with one or other of these shapes. The first figure (figure 3) represents the common relationship, which is a gently curved line. Figure 4 and figure 5 are variants which were less common, but occurred sufficiently often to be of importance.

In order to give an idea of the data from which all the results have been calculated, the actual lysis times of the clots in each of the ten tubes are given in detail in the tables below. The times recorded in table I have been plotted in figure 3, which is a sample of the usual pattern obtained in a majority of patients. 73 patients out of the 101 (73 per cent) conformed to this in greater or lesser degree. Table I itself includes the lysis times from only seven of these patients, such as had a greater number of individual readings (the optimum being a different reading for each dilution), thereby lending themselves to diagramatic representation.

TABLE I

Pattern of lysis times characteristic of the majority.

Name	Age	Diagnosis	Plasma concentration per cent										*
			10	20	30	40	50	60	70	80	90	100	P_{50}
W.B.	42	Repair of perforated peptic ulcer	11	23	23	31	37	37	54	54	71	++	34
J.T.	55	Gastric ulcer	23	28	28	32	32	37	37	47	47	56	32
A.L.	55	Dysphagia	13	24	32	32	48	56	56	71	71	80	48
J.W.	63	Carcinoma anus	13	27	32	32	47	47	47	56	71	96	37
J.E.	65	Head injury	23	23	28	28	48	48	48	56	61	++	35
W.M.	71	Disseminated carcinoma (unknown primary)	9	23	30	48	48	72	72	83	-	++	48
J.G.	81	Jaundice	23	27	47	47	47	51	70	78	98	++	47

unit = hours

* The column P_{50} here and in tables II and III is a "corrected" lysis time for the 50 per cent plasma clot and is explained later.

++ No lysis by 240 hours.

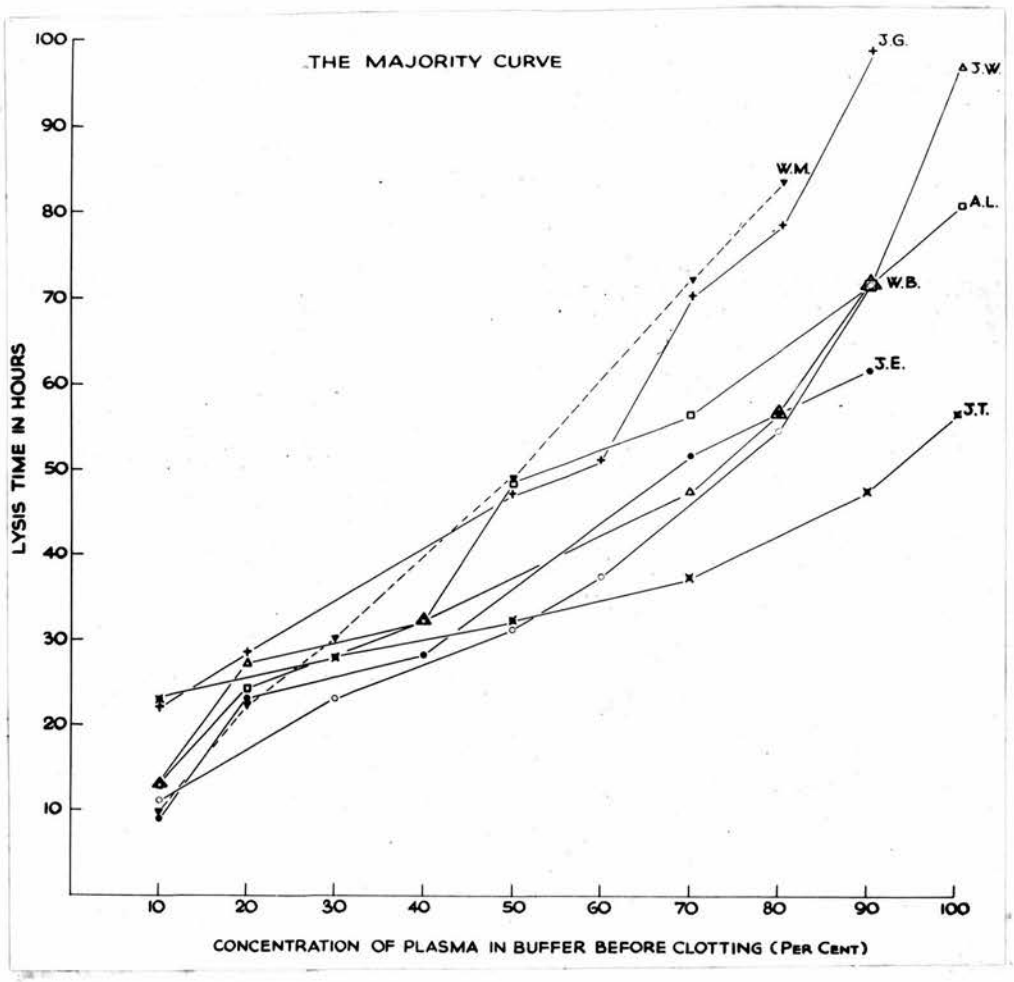


FIG. 3: The lysis time plotted against the initial plasma concentration in seven of the patients where lysis progressed in an orderly fashion from dilute to concentrated plasma clots. Where one observation in the original experiment has recorded for the first time the lysis of two or more adjacent clots (see table I), only the more concentrated of these has been plotted in the figure. This is acceptable because the general trend shows it to be the more accurate of the readings.

Table II supplies the figures from which figure 4 has been prepared and is a fair representation of 21 of the 101 patients (21%).

TABLE II

A common variant of the pattern of lysis times.

Name	Age	Diagnosis	Plasma concentration per cent										P_{50}
			10	20	30	40	50	60	70	80	90	100	
J.B.	29	Jaundice (benign)	47	120	95	80	71	71	71	80	80	80	74
W.J.	67	Carcinoma prostate	28	78	71	71	61	61	61	54	47	47	56
T.N.	72	Carcinoma bladder	47	96	76	76	71	71	56	56	56	56	56
J.P.	73	Carcinoma bladder	55	168	144	127	120	120	120	120	++	++	120

unit = hours

++ No lysis by 240 hours

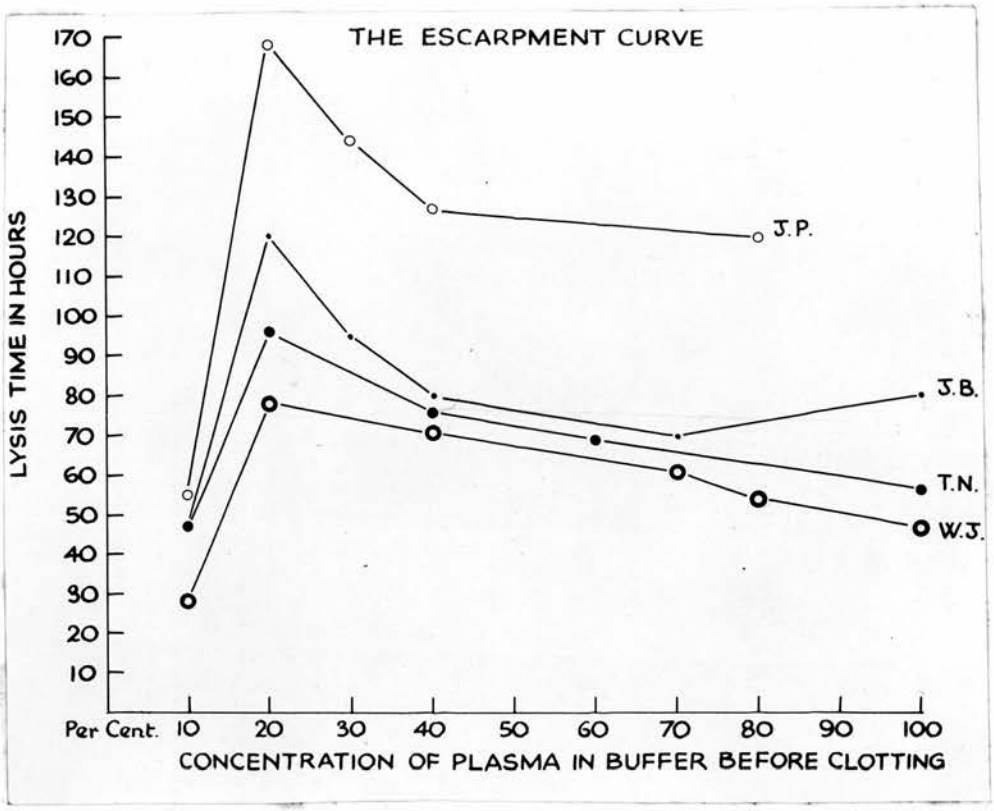


FIG. 4: The lysis time plotted against the initial plasma concentration in four of the patients showing an "escarpment" type of curve. Where one observation has recorded for the first time the lysis of two or more adjacent clots (see table II), only the more recently lysed has been plotted, as judged by the general pattern of lysis. Plasma from W.J. seven days later showed a figure 3 type of curve although the buffer came from the same batch. Many of the patients in this group showed a "rising tail" such as is shown here by J.B. A "rising tail" occurred most commonly when the more concentrated plasma clots had not lysed within about three days, and does not appear to have significance in this method of fibrinolysis assay, being probably an artefact of time and pH. Patient J.B. in this figure and J.T. of figure 3 were examined on the same day, suggesting that the variable factor lay in the patient rather than in the method.

Table III gives the data from which figure 5 has been prepared and represents, in general pattern, about 5 of the 101 patients (5 per cent).

TABLE III

The less common variant of the sequence of lysis times.

Name	Age	Diagnosis	Plasma concentration per cent									P_{50}
			10	20	30	40	50	60	70	80	90	100	
G.T. (1)	55	Carcinoma prostate	144	72	72	55	55	55	55	55	55	72	55
G.T. (2)	56	"	96	96	96	75	72	72	56	56	72	96	72
J.H.	62	Carcinoma bladder	++	++	144	120	99	96	79	99	99	144	99

unit = hours
++ No lysis by 240 hours

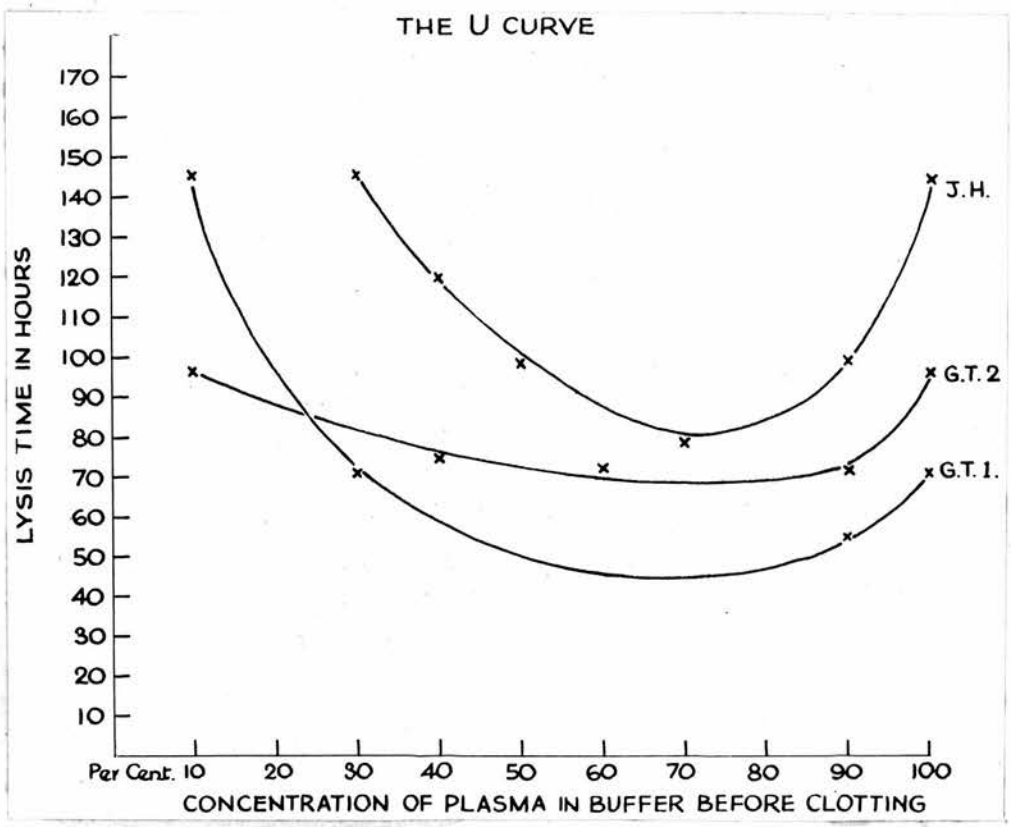


FIG. 5: The lysis time plotted against the initial plasma concentration in two patients with a U-shaped curve where the 10 per cent clot lysed late and adjacent clots lysed earlier in sequence from there. Where one observation has recorded for the first time the lysis of two or more adjacent clots (see table III), only the more recently lysed clot has been used, as judged by the general trend. The "rising tail" is discussed with figure 4. The patient G.T. showed the same phenomenon when re-examined after 14 months, both curves being recorded here. Patient J.H. in this figure was examined on the same day as J.G. of figure 3, the different patterns obtained suggesting that the variable factor lay in the patient and not the technique.

The two patients out of the 101 not yet accounted for had patterns which were unclassifiable as a result of wide tube to tube variations.

In the light of such variables it was difficult to present results from one assay of fibrinolysis which were easy to compare with the results from another, although it should perhaps be stressed that the problem would have appeared simple had a single random dilution of plasma been selected for study. Many figures were prepared on plain and on logarithmic paper, with a view to diagramatic presentation of all results, but none of these was satisfactory. There remained three possible approaches to the problem, all of them using individual lysis times:

1. To follow the lysis time of whole plasma as representing the nearest approach to <u>in vivo</u> blood. This was not feasible because only 63 per cent of the whole plasma clots ever lysed, and they usually did so only after a few days.
2. To follow the lysis time of the 10 per cent plasma clot only. There was much to commend this idea, for it is a dilution used by other workers when presenting results. It has been shown quite clearly here that the 10 per cent lysis time need not be in harmony with the lysis times of even slightly more concentrated samples. Moreover, it can reasonably be claimed that a concentration of plasma which usually lyses within the first 24 hours

must, for accurate work, be observed at regular intervals throughout the 24 hours if a false uniformity of results is to be avoided.

3. To use the 50 per cent plasma lysis time. From a study of all the lysis times and from observation of figures 3, 4 and 5, it was seen how the 50 per cent clot lysis time seemed to be more nearly universally appropriate than any other. It was decided, therefore, to use this lysis time for the presentation of results.

Having decided to present the lysis time of 50 per cent plasma clots in preference to the alternatives, a further problem presented itself. It sometimes happened that the 50 per cent clot lysed during the night when no observations were being made and by morning some adjacent tubes had also lysed. It was difficult to know what time to allocate for the 50 per cent clot itself. Moreover, there was sometimes inconsistency in the smooth progress of lysis from one tube to its neighbour of the next dilution. A formula was therefore devised with the help of the department of statistics in order to smooth off uncertainties and irregularities around the 50 per cent tube.

The formula:

$$P_{50} = L + \frac{(\frac{1}{2} - P_L)}{(P_U - P_L)} \quad (U - L)$$

P_{50} = the lysis time in hours of the 50 per cent clot, corrected where necessary by adjacent figures.

Value L is the observation time in hours such that not more than half the clots have lysed, but had it been the next figure higher more than half would have lysed.

Value U is the observation time in hours, such that not less than half the clots have lysed, but had it been the next figure lower less than half would have lysed.

P_L = the proportion lysed by L

P_U = the proportion lysed by U

The formula is easy to use, most of the figures lending themselves to mental arithmetic after a little practice. The formula contributes nothing where the 50 per cent clot lysis time is unambiguous. In such cases the formula supplies the same figure as when the time is read directly from the results. The ten lysis times of the assay on patient W.B. are reproduced here from table I to serve as an example of the use of the formula.

W.B. 11:23:23:31:37:37:54:54:71:++

$$P_{50} = 31 + \frac{\frac{1}{2} - \frac{4}{10}}{\frac{6}{10} - \frac{4}{10}} \quad (37 - 31)$$

$$= 34 \text{ hours}$$

The columns under the heading of P_{50} in tables I, II and III serve as other examples of the use of the formula.

For the remainder of this work the symbol P_{50} will be used freely without further explanation and in some instances use will be made of the symbolism to describe the whole plasma clot lysis time as P_{100}, and the ten per cent clot lysis time as P_{10} although the formula remains applicable only to P_{50}. While the lysis time P_{50} is regarded as the best single figure with which to present results, the times for P_{100} and P_{10} will sometimes be supplied in addition, out of interest, to allow comparisons to be made. Usually the trends are the same.

Reproducability of the method:

Reproducability studies were carried out on 63 specimens of plasma. Two experiments on each plasma were run in parallel and were set up at the same time using the same buffer. The P_{50} lysis times were listed and a comparison made of the figures from each.

There was very little difference between the figures in each pair. 26 of the 63 pairs of experiments had identical P_{50} lysis times. In the remainder, the experiment set up at the front of the rack lysed more quickly in 25 instances, while the one in the back row lysed more quickly in only 12. The difference between the two rows was between one and four hours, which is too slight to affect the significance of the results. The tendency for the front row to lyse a little before the back row was an impression which was gained early in the work, but it is clearly of little significance. The explanation is not known, but the front row was pipetted before the back row, and

some factor may have influenced the results at that stage.

While the majority of experiments did conform to the pattern of lysis of figure 3, it seemed that the pattern of lysis could vary from patient to patient and from one examination to the next. Enough repetition of work was done on different patients on the same day and on the same patient on different days to suggest that the most important variable factor in the pattern of response lay in the patient and not in the technique, although nothing approaching proof of this can be presented. It was therefore not possible to assume that second assays on the same patient would give the same general pattern of lysis as in the first assay, although this was usually the case. The P_{50} figure nevertheless remained the most valid method of comparing the two.

Fibrinolysis was also assayed by means of fibrin plates. Only a restricted investigation was made using this technique, and such modifications as were made to the method of Astrup and Müllertz (1952) are given in the appropriate section devoted to "Experiments on fibrinolysis using fibrin plates" in chapter three.

2. PLASMA FIBRINOGEN ESTIMATION

Almost all the fibrinogen estimations in the control series have been by recalcification of plasma and isolation of fibrin clot according to Cullen and van Slyke (1920) followed by Kjeldahl digestion and distillation. Many of the

plasma samples from patients suffering from carcinoma of the prostate were estimated by the rapid turbidometric method of Podmore (1959) using sodium sulphate and a grey wedge photometer, although usually the longer method was employed on the same specimen.

During the course of this work there were two types of circumstances where plasma fibrinogen estimation presented peculiar problems.

Fibrinogen estimation in the presence of acute hypofibrinogenaemia.

When the plasma fibrinogen falls catastrophically, as in patient E.S. (chapter four, case 1), and to a lesser extent in patient J.H. (chapter six, case A), or much more commonly, where one fears that the fibrinogen level may have fallen, because the patient is bleeding profusely and the blood is not clotting normally, then a rapid method of fibrinogen estimation is not only desirable but is essential. The patient's life may depend on a rapid and accurate diagnosis of the clotting defect. With this need in mind, rapid methods of fibrinogen estimation were reviewed in the literature and two were selected at first, as being easy to perform in an emergency without prior preparation. The turbidometric method of Podmore and allied methods, of which there are several, were not at first considered suitable for such very rare emergency use, although ultimately they were adopted.

The first method which was chosen is qualitative only and involves the adding of 0.2 ml. of commercial thrombin

solution (made up to 50 units per ml.) to one or two ml. of whole blood freshly removed from a vein. If there is no obvious clot within one minute, fibrinogen deficiency is to be suspected. The same test performed with whole plasma from oxalated blood takes a little longer to perform but is very much easier to read and interpret, and is to be preferred. A normal control is advised in order to demonstrate the potency of the thrombin and size of a normal clot.

The second method which was chosen is quantitative in a rough way, and can be performed in any laboratory possessing thrombin and a centrifuge. This is the method recommended by Wolf (1954). Oxalated (not heparinised) plasma from the patient and a control is diluted to one part in ten with saline. 2 ml. of this are placed in the first of a row of six test tubes, and doubling dilutions in saline made in successive tubes down to 1:320, the final volume in each tube being 1 ml. To each tube 0.1 ml. of thrombin using 50 N.I.H. units per ml. is added. Clotting is practically instantaneous. No more than a minute need elapse before each tube is examined in front of a good light for the presence or absence of clot. The smallest clot is difficult to see, whether this is from the patient or the control. It is a disadvantage of the method. For those familiar with the wisp which is the smallest clot, the results are reasonably accurate. The smallest visible clot is usually the result of clotting a solution containing approximately 0.001 g. fibrinogen per 100 ml. The following table shows some of the work done using the Wolf test. Only

TABLE IV

Some results using Wolf's (1954) method of fibrinogen estimation.

Name	Plasma dilution in saline						Fibrinogen level g./100 ml.	
	1:10	1:20	1:40	1:80	1:160	1:320	Wolf estimate	Kjeldahl
Control A	++	++	++	+	+	+	-	0.32
B	++	++	++	+	+	+	-	0.28
C	++	++	++	+	+	+	-	0.23
D	++	++	++	+	+	+	-	0.28
E.S. (emergency)	+	+	-	-	-	-	(0.012 - 0.025	-
Control	++	++	++	+	+	+		-
E.S. (12 hours after fibrinogen i.v.)	++	+	+	+	-	-	(0.05 - 0.08	0.08
Control	++	++	++	+	+	+		-
E.S. (3)	++	++	+	+	+	-	Normal	0.39
Control	++	++	++	+	+	-		-
E.S. (4)	++	++	++	+	+	+	Normal plus	0.62
Control	++	++	++	+	+	?		0.18
E.A.S.	++	++	++	+	+	+	Normal	0.49 ≡
J.H.	++	+	+	+	±	-	0.10	0.13

++ and + = clot ± = uncertain clot - = no clot

≡ turbidometric method. The clot prepared for fibrinogen estimation by Kjeldahl method lysed completely.

those tests are recorded where a second assessment of fibrinogen concentration was made by another method, or where tests in parallel gave helpful information.

Although symbols are given for the size of clot, these are ignored in the assessment. Only the presence or absence of clot is of significance. The normal range for plasma fibrinogen is taken to be 0.2 - 0.4 g. per 100 ml. and the tubes are in doubling dilutions. Consequently a difference of two tubes between the last clot in the patient's row of tubes and the control means a difference in fibrinogen content to a factor of four. The second last column of table IV shows that the method often works out fairly accurately, even in an emergency.

Fibrinogen estimation in the presence of active fibrinolysis.

When the plasma clot from patient E.A.S. was prepared for estimation of fibrinogen by the Kjeldahl method, it appeared normal. The clot was then left at room temperature as is customary using that method, but in the meantime it lysed. An aliquot of the same specimen had already been examined using a turbidometric method and the fibrinogen content was 0.49 g. per 100 ml. Because of the impossibility of preserving the clot in the presence of lysins active at room temperature, without modifying the locally standardised laboratory method for such an investigation, it was felt that a turbidometric

method of estimation of fibrinogen should always be available for emergency use, however infrequently this might be called upon. The equipment required is simply maintained - a Medical Research Council grey wedge photometer, a 10.5 g. per cent sodium sulphate solution and a standard graph from which to calculate results (Podmore, 1959). Although most of the patients in the main series had plasma fibrinogen estimations by both methods, there was no subsequent instance when the clot was recognised to lyse before the Kjeldahl estimation could be performed. The results of turbidometric estimations have therefore been omitted because they ran the same pattern as by the Kjeldahl method and the figures themselves were almost the same. Moreover most of the control series had plasma fibrinogen estimation by Kjeldahl method only.

3. HAEMOGLOBIN ESTIMATION

The haemoglobin concentration was measured using a Medical Research Council grey wedge photometer. 100 per cent = 14.8 g./100 ml. (King, Wootton, Donaldson, Sisson and Macfarlane, 1948).

4. PLATELET COUNT

Platelets were counted by Lempert's (1935) method. Normal range from 150,000 to 350,000/c.mm.

5. QUICK "PROTHROMBIN" TIME

The technique used here has been described and discussed by Quick (1942). Human brain thromboplastin and 0.025 M. calcium chloride was used. The anticoagulant was the Heller and Paul (1933-34) mixed oxalate, evaporated to dryness.

CHAPTER THREE

THE CONTROL SERIES

Fibrinolysis related to age and other factors.

INTRODUCTION

The control series was gathered together to provide information with which to compare changes found in a series of patients suffering from carcinoma of the prostate. A preliminary study of the figures in the early stages of the prostate series had shown wide variations from one patient to another and it seemed very likely that only falsely justified conclusions would be reached unless a large control series was prepared.

The problem of selecting the controls was not straightforward. It was difficult to decide what comprised a control for a patient suffering from carcinoma of the prostate. Patients suffering from benign hypertrophy of the prostate had much to commend them as controls. They were of similar age and the same organ was affected. However, it has been claimed that histological malignancy can be demonstrated in a high proportion of elderly men not suspected to be suffering from carcinoma (Franks, 1954). Moreover, it was felt that only patients in the ward should be included because of the known influence of exercise and excitement on fibrinolysis such as would more likely affect out-patients. This largely restricted available cases of benign hypertrophy of the prostate to emergency admissions with acute retention of urine, or to post-operative cases, neither of which were ideal. It was decided therefore to accept cases of benign hypertrophy of the prostate as they arose, but in addition to seek out

approximately equal numbers of men of all ages, the one group having been admitted to hospital because of a malignant condition, the other group because of a non-malignant condition. As far as possible days of stress for the patient were avoided and no sample was taken within a day or two after surgery without due note being made.

METHOD

All patients were examined for fibrinolysis by the same method, using serial dilutions of plasma in buffer, followed by observation for the times of clot lysis. This method has been described in detail already. Blood samples were taken from the patients near 10 a.m. in almost all cases and all patients had eaten a hospital breakfast. Specimens were normally taken only on a Tuesday or a Wednesday of each week.

CHOICE OF PATIENTS

There were 138 patients in the control group. 21 of these had been admitted to hospital because of benign hypertrophy of the prostate and these have been treated as a separate group. Most of the remaining 117 controls were allocated for the purpose by house surgeons, whose choice was fairly random and was quite independent of the question of fibrinolysis. 62 of these had been admitted to hospital because of a variety of conditions, mainly surgical in interest

but none of them malignant. The remaining 55 were known to be suffering from a malignant condition elsewhere than in the prostate. Patients were commonly chosen who had been admitted to the ward within the previous few days but the day of admission itself was avoided. Almost all the 83 patients in the non-malignant group were in a general surgical ward, while almost all the 55 patients in the malignant control group were in a radiotherapy ward. Only the first examination for fibrinolysis on each patient has been accepted for this part of the study.

RESULTS

OBSERVATIONS SUBJECTED TO STATISTICAL ANALYSIS

The influence of age on fibrinolysis

Little would be gained by presenting the lysis times of all the ten tubes of each experiment for the reasons presented in the last chapter. Therefore only the lysis times of the 50 per cent clots (P_{50}) have been supplied. These have been listed for all 138 patients in table XXXII in appendix 2. The 101 patients whose patterns of results were discussed in chapter two are included in this table, except those suffering from carcinoma of the prostate, who are included in table XXXIII in appendix 2. The three sub-groups of the control series have been tabled separately, as benign prostatic hyperplasia, non-malignant miscellaneous disorders and non-prostatic malignant miscellaneous disorders.

When the lysis times were arranged in sequence according to the age of the patient, it became apparent at once that there was probably an effect of age. The figures were examined for their statistical significance.

The lysis times were taken by decades, as they have been presented in table XXXII in appendix 2, and the mean lysis time for each decade has been plotted against the mean age, in figure 6. Each of the three sub-groups of the control series taken separately and then together shows an increase of lysis as age progresses.

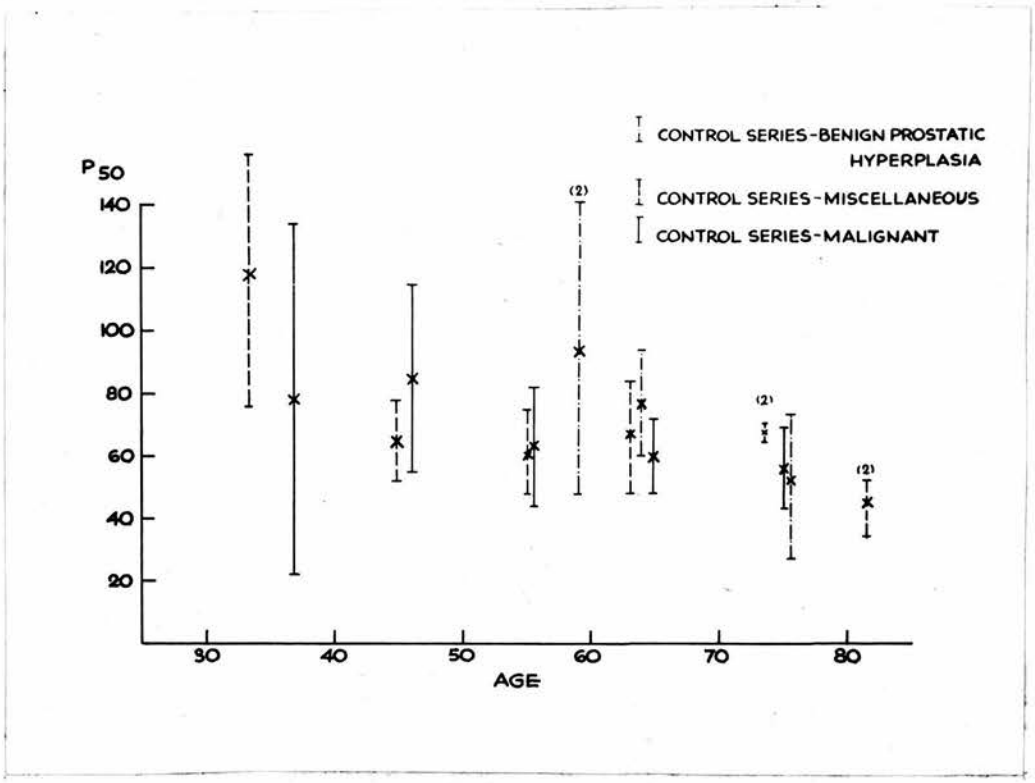


FIG. 6: The relationship of age in years to the lysis times (P_{50}) for the three sub-groups of the control series. The mean age for each decade has been plotted against the mean lysis time together with two standard errors on either side. The downward trend of the mean lysis time as age progresses is apparent.

An analysis of results from the carcinoma of prostate group will be presented in the next chapter. It is necessary to anticipate this to the extent of stating that the carcinoma of prostate series shows the same increased rate of lysis with age as is shown by the control group. Therefore all three sub-groups of the control series and the main series of carcinoma of prostate have been taken together to prepare the figures plotted in figure 7. These points form virtually a straight line, particularly from the age of 40 onwards, where there were more cases in each decade. This shows clear evidence of an increased rate of lysis with age.

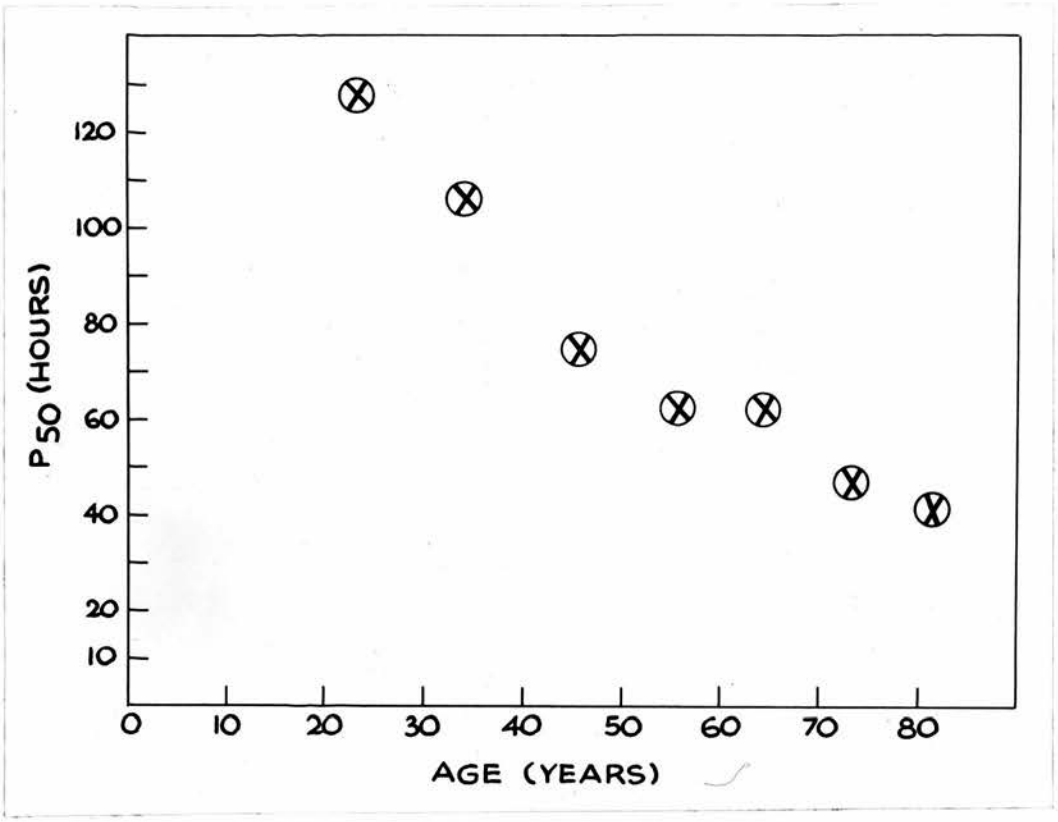


FIG. 7: The relationship of age in years to the lysis times (P_{50}) for the entire control series and the main series of carcinoma of prostate, treated as a single group. The mean age for each decade has been plotted against the mean lysis time. The increased rate of fibrinolysis with age is apparent.

The comment made on the statistics is as follows. "In each series there is a marked tendency for the average P_{50} to decrease with age. This is too obvious to require significance testing, particularly as it occurs in each series in exactly the same way. The average P_{50} level (of the carcinoma of prostate series) is lower than that in the control groups, but this effect is no greater than would be expected because of the higher average age of the carcinoma of prostate group.

"There is no suggestion of departure from normality of distribution (which might require examination of minimum values as well as the means) and so it can be concluded that the groups do not differ in any significant respect.

"The only suggestion of a difference is in the 70-80 age-group, but as this is not borne out by differences in the age-groups either above or below it may be assumed to be a chance effect. It would certainly not be significant in the conventional sense."

Reference will be made again to these results in the main discussion which follows chapter six. There some of the broader issues of fibrinolysis will be discussed.

SOME OTHER INVESTIGATIONS CONCERNING FIBRINOLYSIS AND FACTORS CAPABLE OF INFLUENCING THE RESULTS.

The effect of adrenalin and excitement

A normal male control aged 38 volunteered to be the subject of an investigation into the effect of adrenalin on fibrinolysis. On two previous occasions his ten-dilution fibrinolysis experiment had failed to lyse in any tube, even by 240 hours. On the morning of the experiment, to which he was not looking forward, the lysis time can be seen in table V to have shortened considerably. Eight minims of adrenalin 1:1000 were then injected slowly into the deltoid muscle and a second blood sample removed several minutes later. This was at the period of maximum discomfort, when a minor headache had developed, pupils dilated and pulse increased from 60 per minute to 80 per minute. An identical fibrinolysis assay was performed on the second specimen.

TABLE V

Two base-line fibrinolysis assays, followed by one during mild anxiety and finally one during the unpleasant effect of an adrenalin injection.

	Lysis time P_{100}:	P_{50}:	P_{10}	Fibrinogen g./100 ml.
Previous assay 1	++:	++:	++	-
Previous assay 2	++:	++:	++	-
Before adrenalin	++:	36:	13	0.27
After adrenalin	70:	14:	6	0.28

++ No lysis by 240 hours

Although the number of times the assay procedure was practised numbers several hundred, and the venepuncture was almost invariably performed by the same person, there was ordinarily no way of assessing the anxiety felt within the patient at the time of the sample. There was, however, one man aged 60 who had been admitted on the preceding day with a view to operation for varicose veins. Before, during and after the venepuncture he was talking with much nervous anxiety about the progress of the professorial ward round which was slowly approaching his bed. It was clear that he was unusually anxious at the time of the venepuncture. His lysis time (P_{50}) was only 15 hours and his whole plasma clot had lysed by 29 hours, which is faster than usually occurs.

Comment: The findings in both these subjects may be used in support of published work, notably that of Biggs, Macfarlane and Pilling (1947). Adrenalin and anxiety both appear to increase the fibrinolytic activity of the blood. The effect of anxiety is presumably mediated by adrenalin.

In the context of this work, these experiments, together with others not quoted of less certain authority, give a measure of the change which can be demonstrated by the ten-dilution assay method, without it necessarily reflecting pathological conditions. They give a reminder that controls for fibrinolysis experiments are difficult. Even a normal person, being excited, can give quite a different result from the same person mentally at rest.

The immediate effect of surgery on fibrinolysis

Table VI shows the effect of surgery on fibrinolysis as shown in two patients diagnosed as suffering from carcinoma of the prostate.

TABLE VI

The immediate effect of surgery on fibrinolysis

Name	Age	Operation	Fibrinolysis preoperative $P_{100}:P_{50}:P_{10}$	Fibrinolysis postoperative $P_{100}:P_{50}:P_{10}$	Time of specimen after operation
F.P.	69	Trans-urethral resection	++:103: 71	20: 16:<16	25 min.
T.L.	64	Bilateral orchid-ectomy	71: 66: 25	16: 14:<14	15 min.

Lysis time in hours

Comment: This table shows that one of the immediate effects of surgery can be a considerable increase in the rate of fibrinolysis. Macfarlane's (1937) observation has thus been repeated. In the context of this work it can be pointed out that very rapid fibrinolysis, such as is associated with surgery, can clearly be present without any unusual clinical bleeding.

The effect of heparin on the fibrinolysis assay

A man aged 54 was in hospital for investigation of a vascular disorder. A ten tube dilution assay was set up in the morning immediately preceding an intravenous injection of

10,000 international units of heparin. Forty minutes after the heparin injection, when the whole blood clotting time was more than 30 minutes, the fibrinolysis assay was repeated. The results are shown below.

P_{50} before heparin = 55 hours

P_{50} after heparin = 66 hours

Comment: There does not appear to be any significance in the difference here. In the presence of an obvious increase in the whole blood clotting time, it is reasonable to assume that if heparin, given as here, does cause any difference in the lysis time such a difference must be small.

Fibrinolysis related to the plasma fibrinogen level

A man aged 66 who died four days subsequently of carcinoma of the bronchus had P_{50} lysis times of ten and nine hours on two assays made in parallel on the same specimen. These lysis times are faster than was ordinarily found, yet his plasma fibrinogen level was 1.06 g. per 100 ml. Clearly the high fibrinogen level in this patient did not stand in the way of fast fibrinolysis. Adelson and Roeder (1958), however, appeared to show that the fibrinogen level bears an inverse relationship to the fibrinolysis present in the sample. In view of the apparent importance of this point, lest it be used as a criticism of the method used here and of conclusions drawn from it, the following table was prepared. All the 12 examples in the main series and its controls, who had a plasma fibrinogen

level greater than 0.80 g. per 100 ml. have been tabled with their P_{50} lysis times. Similarly the 12 lowest plasma fibrinogen levels (range from 0.17 to 0.26 g. per 100 ml.) have been listed with their lysis times.

TABLE VII

The lysis times associated with the 12 greatest fibrinogen levels and with the 12 least fibrinogen levels

Name	Age	Diagnosis	Fibrinogen g./100 ml.	Lysis time (P_{50}) hours
G.C.	83	Sarcoma of thigh	1.16	79
H.W.	66	Carcinoma of bronchus	1.06	10
G.F.	68	Acute cholecystitis	1.00	144
W.W.	76	Carcinoma of prostate	0.98	166
F.G.	76	Carcinoma of prostate	0.94	86
R.Y.	63	Multiple infections	0.90	++
T.G.	68	Carcinoma of prostate	0.90	52
H.A.	64	Carcinomatosis	0.89	43
B.S.	67	Carcinoma of bladder	0.88	96
W.M.	61	Gastro-entero-colic fistula	0.86	176
T.M.	40	Resection gastric ulcer	0.81	95
R.L.	79	Carcinoma of nasal antrum	0.81	47
T.H.	82	Paget's disease	0.17	39
E.R.	61	Chronic granulocytic leukaemia	0.18	48
F.W.	68	Carcinoma of bladder	0.20	65
I.H.	71	Carcinoma of prostate	0.21	++
G.M.	30	Anxiety	0.23	118
O.H.	69	Carcinoma of prostate	0.23	69
T.S.	38	?	0.24	165
J.L.	38	Incisional hernia	0.25	105
A.T.	67	Carcinoma of prostate	0.26	68
J.D.	73	Carcinoma of prostate	0.26	7
H.P.	44	Carcinoma of prostate	0.26	120
J.T.	78	Benign prostatic hyperplasia	0.26	67

These points have been plotted on a scatter diagram figure.

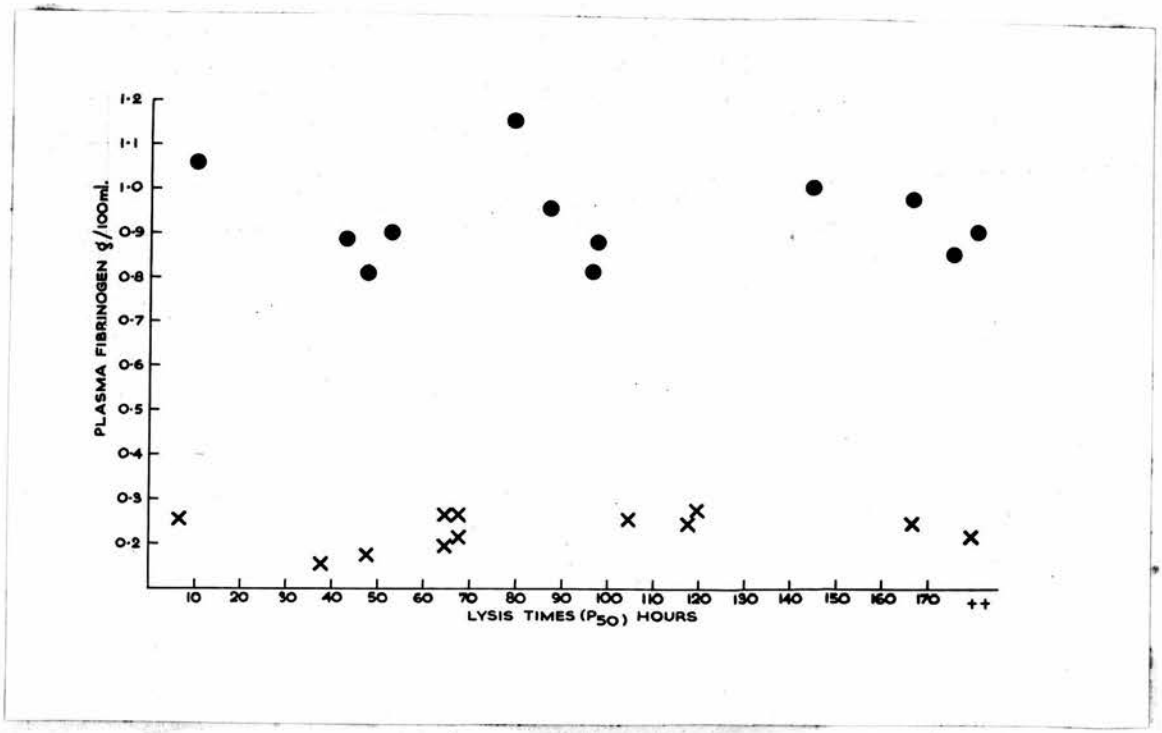


FIG. 8: The relationship of 12 high plasma fibrinogen levels to the lysis times associated with them, and of 12 lower fibrinogen levels similarly related.

Comment: It can be seen from the general arrangement of the points that when the fibrinogen level is unusually high some clots lyse quickly. Similarly when the fibrinogen level is low, or low normal, some clots lyse slowly. There seems to be no evidence here to suggest that high fibrinogen levels inhibit lysis or that the method used masks lysis at high fibrinogen levels.

The influence of sex on fibrinolysis

Apart from the group in table VIII, all the patients examined have been men, because the series has concerned carcinoma of the prostate and the controls required for this. The following table shows the plasma lysis times found in seven women whose blood was examined incidentally to the main series.

TABLE VIII

The lysis times in seven women of different ages and diagnoses

Name	Age	Diagnosis	Lysis time (hours) P_{100}:P_{50}:P_{10}
B.B.	26	Capillary defect	97: 94: 94
A.C.	46	Acquired deficiency of antihaemophilic factor	47: 47: 23
E.H.	52	Biliary cirrhosis	++: 68: 68
E.B.	55	Functional disorder	++:117: 26
F.E.	60	Metastatic carcinoma	93: 93: 93
E.B. (1)	65	Cirrhosis of liver	++: ++: 90
(2)	"	" " "	66: -: 42
A.H.	71	Retinal bleeding	++: ++: 72

Comment: The figures in this table can be compared with those in the control series in figure 6. There seems to be no clear-cut difference between the two sexes but the number of cases is far too small and the method insufficiently refined for any conclusion to be possible about lesser differences. Such insignificant differences as are shown tend towards a longer lysis time in women. In a series of 30 men and 30 women between the ages of 18 and 40, Fearnley and Tweed (1953) also found a somewhat longer lysis time in the women compared to the men, but doubted whether the difference was significant.

The effect of calcium on fibrinolysis

Although it will be shown that calcium in the buffer has not materially affected the results in the main series of patients, this is not to imply that calcium plays no part in fibrinolysis. Many of the early experiments where calcium was used showed a delayed lysis, particularly of the more dilute plasma clots. Delayed lysis of dilute plasma clots can also occur using veronal buffer at pH 7.4 even in the absence of calcium, as has already been shown, but the frequency of its occurrence with calcium appears to be greater.

Two experiments are presented below to demonstrate differences which are claimed to be due to the calcium present in the buffer. The two assays on each patient were set up simultaneously using the same specimen of plasma but different buffers, each a veronal buffer at pH 7.4, but one containing added calcium chloride.

TABLE IX

The inhibitory effect of calcium in two experiments.

Name	Age	Diagnosis	Lysis time with calcium (hours) P_{100}:P_{50}:P_{10}	Lysis time without calcium (hours) P_{100}: P_{50}: P_{10}
A.C.	63	Inguinal hernia	++: 71: 47	++: 49: 23
E.M.	56	Peptic ulcer	++: 51: 23	++: 31: 7

++ No lysis by 240 hours

Comment: Although the use of calcium has not infrequently been recommended in techniques for demonstrating fibrinolysis, analysis of the part it plays has usually shown it to be inhibitory to fibrinolysis. Fearnley and Tweed (1953) demonstrated the inhibitory effect of calcium although they continued to use it subsequently (Fearnley and Lackner, 1955). Medart (1958) found that calcium, magnesium, manganese, cobalt and zinc were approximately equally effective in reducing the rate of proteolysis by trypsin. The effect may be by a protective action on the fibrin rather than by an inhibition of the plasmin. The two experiments which have been shown in table IX, in addition to many others which were less well controlled, all support the evidence that calcium is inhibitory to fibrinolysis.

Experiments on fibrinolysis using fibrin plates

Experiments were carried out on plasma from patients without known malignant disease in order to assess some of the claims made for the fibrin plate technique. Plates were

prepared by a method which was largely that of Astrup and Müllertz (1952) but bovine fibrinogen as supplied by The Armour Laboratories (at that time Armour and Company Ltd.) was used in place of freshly prepared ox fibrinogen. Nine ml. of fibrinogen solution in buffer at pH 7.8 were poured into each of several petri dishes and were clotted with 0.8 ml. of thrombin (Maw) 50 N.I.H. units per ml. This made a thin smooth layer of fibrin in each dish.

The Armour fibrinogen contained only 40 - 50 per cent of clottable protein and also contained 40 - 50 per cent by weight of sodium citrate. Presumably because of difference in preparation it was found that a concentration of 0.8 g. per 100 ml. of this bovine fibrinogen was more suitable than the recommended 0.1 - 0.2 g. per 100 ml. of freshly prepared ox fibrinogen. Buffer was prepared by adding to 66.2 ml. of 0.1 M. sodium diethyl barbiturate, 33.8 ml. of 0.1 M. hydrochloric acid and 32 ml. distilled water. Streptokinase (Burroughs Wellcome and Company) contained 5,000 Christensen units per 1 ml.

When plates were to be heated in order to inactivate plasminogen, it was found to be satisfactory to leave them at 90°C for 20 minutes. Drops of the solution to be tested were always of 0.03 ml. and were placed carefully on the fibrin plate. The area of lysis was measured by multiplying diameters at right angles after an appropriate incubation period at 37°C, usually 24 hours.

Figure 9 illustrates the results of one experiment designed to test the relative effects on heated fibrin plates of streptokinase by itself, bovine fibrinogen by itself (although containing such fibrinolytic factors as were not excluded by the fractionation), a mixture of both, and trypsin.

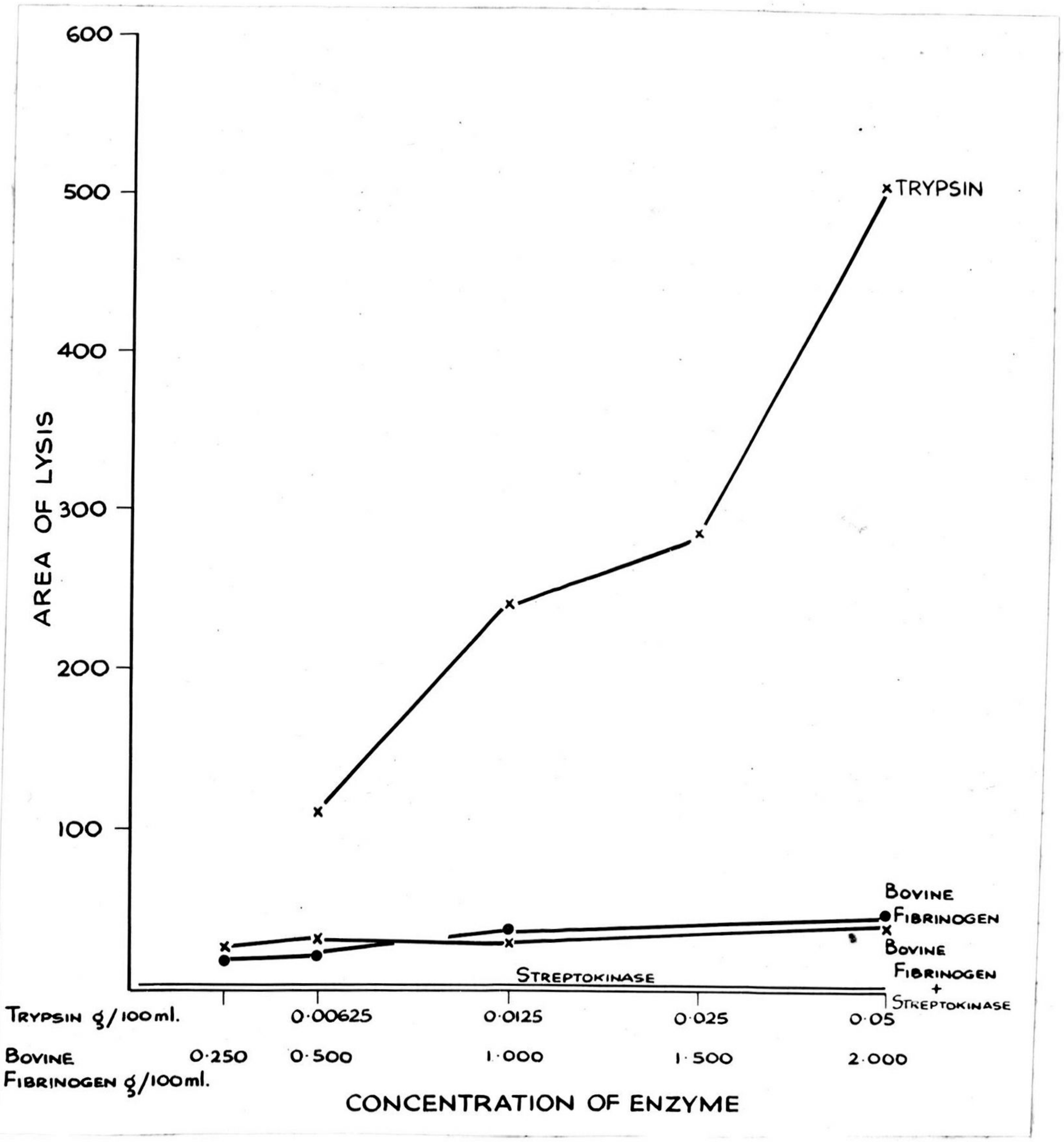
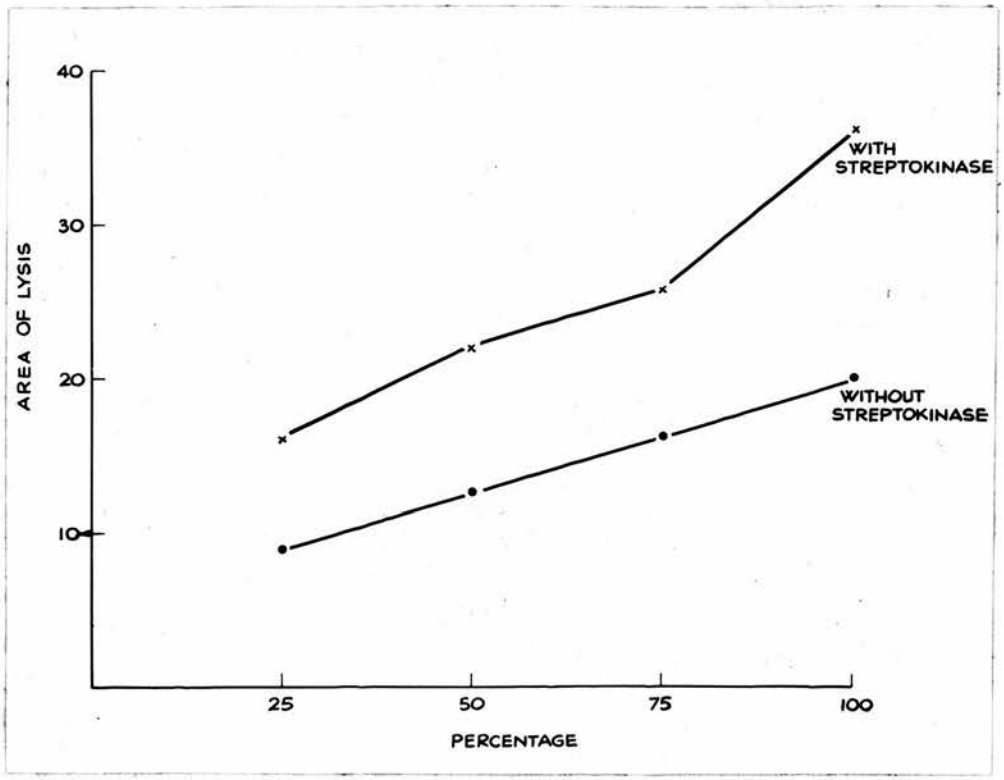


FIG. 9: Serial dilutions of various substances on heated fibrin plates after incubation for 24 hours. The unit of area is arbitrary.

Observations:

1. Unheated bovine fibrinogen had almost negligible spontaneously-activated plasmin activity on the heated plate.
2. Streptokinase itself had no lytic activity and did not appear to activate bovine fibrinogen.
3. The trypsin effect, used here as a positive control, was considerable.

Figure 10 illustrates the effect of streptokinase on human plasma using unheated fibrin plates. Fresh oxalated plasma was obtained from two patients in hospital as a result of non-malignant disorders. Four dilutions of each were made in saline. The same concentration of streptokinase was used as before. The results from the two patients were similar and therefore only one set has been recorded.



The horizontal scale represents percentage of plasma.

FIG. 10: The effect of incubating four dilutions of the same human plasma on an unheated fibrin plate and of adding a constant amount of streptokinase to each dilution of plasma. The unit of area is arbitrary.

Observation: Although there appears to be definite plasmin activity in the plasma of the spontaneously-activated type, this activity has been increased by the addition of streptokinase.

Figure 11 illustrates the relative effect on unheated fibrin plates of a euglobulin prepared from pooled human plasma according to the method of Milstone (1941); of trypsin; and of streptokinase, in the same concentration as used above.

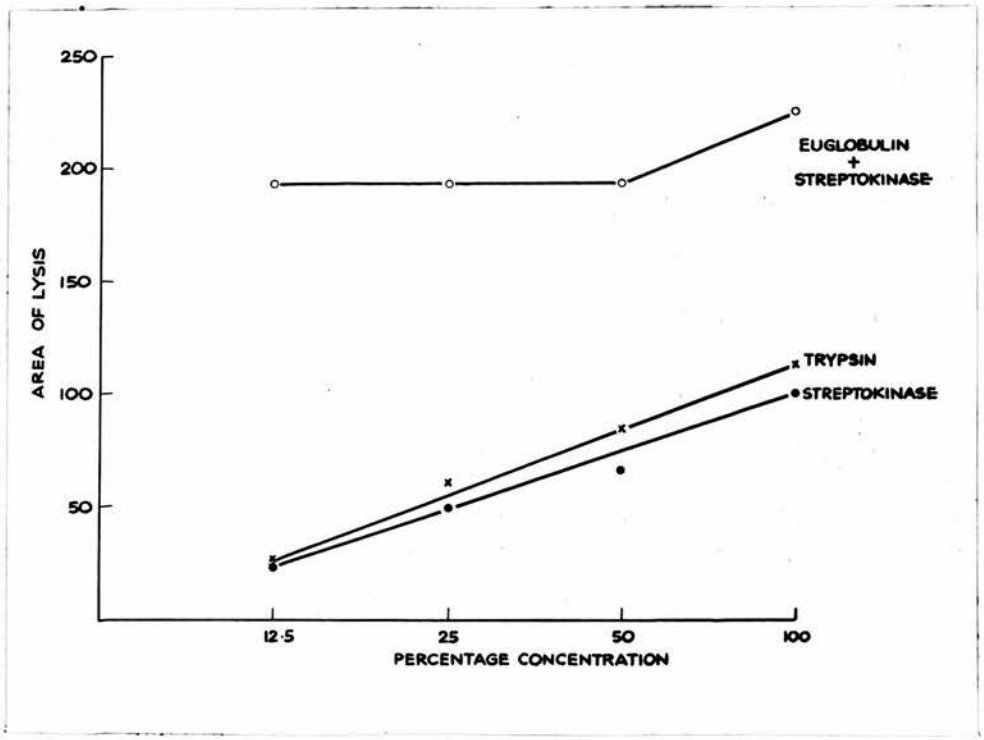


FIG. 11: Trial of a preparation of euglobulin compared with one of trypsin. The unit of area is arbitrary.

Observations and Conclusions: It is clear that Milstone's euglobulin, activated by streptokinase, can be an extremely powerful lytic agent. Very little importance can be placed on the relative strengths of the substances used, as illustrated in the figures, because further analysis of varying concentrations was not undertaken. Note that the effect of streptokinase on unheated bovine fibrin was greater than that of streptokinase with bovine fibrinogen on heated fibrin (figure 9). The apparent effect of streptokinase on an unheated bovine fibrin plate is itself of interest.

The fibrin plate method is clearly one of importance. Difficulty was experienced, however, in obtaining sharply defined areas of lysis on the plates. It was felt that the method itself demanded special study before results of value could be obtained from it. The method was therefore not used in parallel with that of the lysis time of formed clots.

CHAPTER FOUR

THE MAIN SERIES

The influence of prostatic carcinoma on fibrinolysis.

INTRODUCTION

If in fact prostatic carcinoma leads to an increased rate of fibrinolysis, as claimed by Tagnon, Whitmore, Schulman and Kravitz (1953), this may be demonstrated in several ways. The mean of a whole series of assays will show the difference, provided most of the patients have an increased rate of lysis; or if the majority lyse normally, but there are enough individual examples of increased lysis, this also will weight the mean as will be apparent in a distribution figure. If there is an all-or-none mechanism and the incidence of abnormal lysis is very low, then the mean may not show a difference from the controls, and yet individual examples of abnormal lysis may still exist.

Choice of patients

There were 68 patients in the main series. All of them were suffering from carcinoma of the prostate. The diagnosis was proven histologically in 44 and was beyond all reasonable doubt, although without histological proof, in 17. The diagnosis in the latter was supported by the presence of osteoplastic secondary deposits in bones, or raised serum acid phosphatase levels, in addition to the characteristic findings on palpation of the prostate. In seven it was a clinical diagnosis made on the history and after rectal examination, without strong enough supporting evidence to make the diagnosis unchallengable. These points of difference have been shown in table XXXIII in appendix 2. The three sub-groups were treated

separately in the analysis but no difference was found and they are therefore being treated here as a homogeneous group of carcinoma of the prostate. The patients are unselected by as much as no patient was refused inclusion in the series where the opportunity of seeing him was known, and this covers approximately three and a half years from 1956 to the middle of 1960. The group is not a true random selection, however, because notice of patients was invariably by courtesy of members of the hospital staff, and this led to a majority of patients arising from two surgical units which had a special interest in this type of case. Two patients (to be described as case 1 and case 2) were examined in the first place because of a bleeding episode, and in this sense were selected. The remaining 66 patients were examined as part of a research investigation into carcinoma of the prostate, without special regard to bleeding, and in this sense were unselected. A few patients in each decade of age were examined of necessity as out-patients and were included in the group, but can be identified in table XXXIII in appendix 2. Although multiple examinations were made on many patients, only the first assay has been used for the main analysis.

Method

All patients were examined for fibrinolysis by the same method, using serial dilutions of plasma with buffer, followed by observation for the times of clot lysis. This is the method already described, and used in the control series.

It was not possible to take blood samples at such regular times of the day or of the week as in the control series but the majority of specimens were taken around 10 a.m. Calcium buffer was used in 23 assays and the effect of this is discussed separately.

RESULTS

OBSERVATIONS SUBJECTED TO STATISTICAL ANALYSIS

The times of lysis as obtained from assays on all 68 patients have been included in table XXXIII in appendix 2. The mean lysis time (P_{50}) for each decade, together with the range included within twice the standard errors in either direction, has been shown in figure 12. This has been superimposed on the results from the control series, as shown already in figure 6.

Because the results from the control series have already been presented, part of the results from the carcinoma of prostate series have had to be anticipated. This has arisen because both the control series and the main series showed a definite speeding of lysis with age. Moreover the three sub-groups of the control series did not differ within themselves in this respect nor with the main series of carcinoma of the prostate. Therefore all groups were taken together in the preparation of figure 7, which shows clear evidence of an increasing rate of lysis associated with increasing age.

It was noted that a greater proportion of patients in the carcinoma of prostate group had been examined when

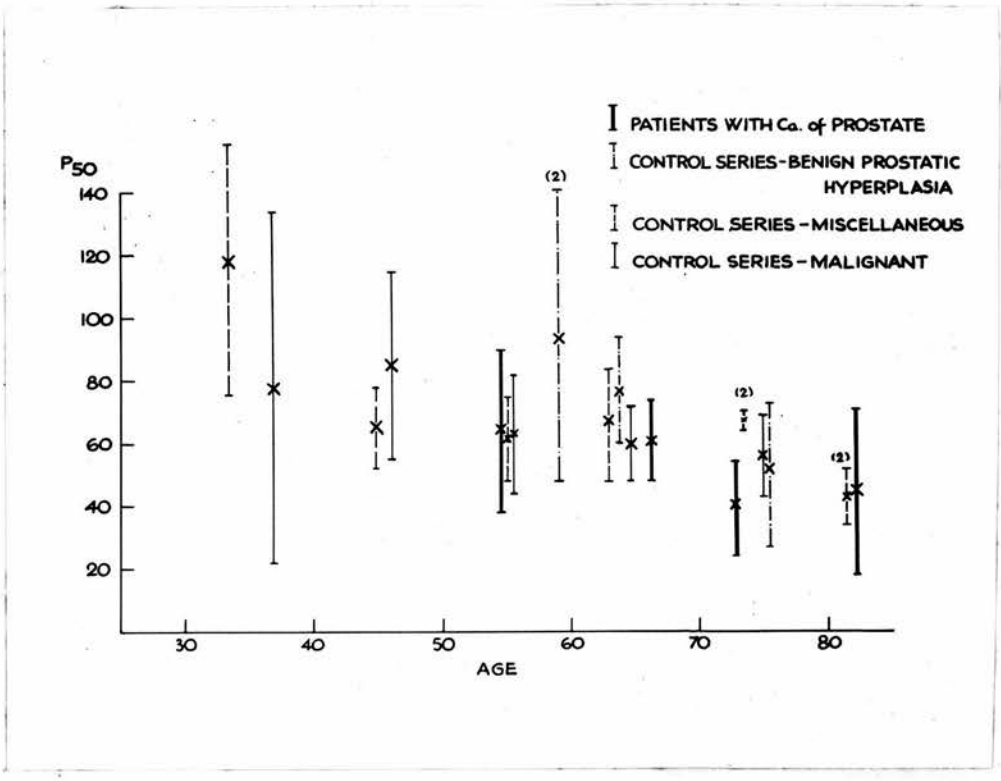


FIG. 12: The relationship of age in years to the lysis times (P_{50}) for the main series of carcinoma of prostate superimposed on those for the control series. The mean age for each decade has been plotted against the mean lysis time together with two standard errors on either side. The downward trend of all groups as age progresses is apparent.

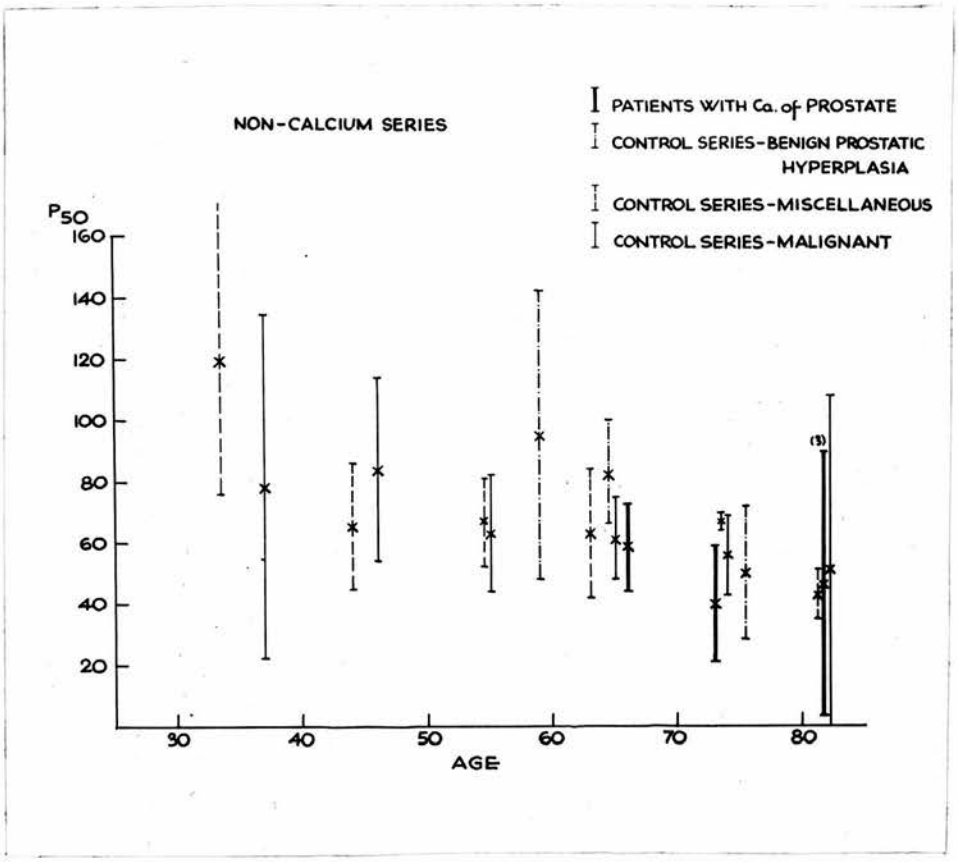


FIG. 13: The relationship of age in years to the lysis times (P_{50}) for all groups, excluding assays where calcium was used in the buffer. The general trend is the same as in figure 12.

calcium buffer was in use than had been the case in the control series. Figure 13 was therefore prepared from all the results of the main series and the control series, excluding all assays which had been made using calcium buffer. This figure can be compared to figure 12 visually and also by statistical assessment. Calcium made no material difference to the conclusion. Calcium thus seemed to play no significant part in delaying lysis in these groups of experiments as measured by the lysis time of the 50 per cent clot.

It was apparent on statistical examination that the average P_{50} level in the carcinoma of prostate group was lower than that in the control groups, but this was no greater than would be expected because of the higher average age of the carcinoma of prostate group. In addition there were 13 out-patients in the main group. All that was left, therefore, was to see if there was an incidence of abnormal lysis in a small proportion of patients, insufficient to weight the mean significantly. It was noted, however, in the statistical analysis, including observation of scatter diagrams prepared for all groups, that there was no suggestion of departure from normality of distribution such as might require examination of minimum values as well as the means. The scatter diagrams have not been included, because they do not contribute anything positive in themselves and the constituent points have already been recorded in tables XXXII and XXXIII in appendix 2 and have been incorporated into figure 6 and figure 12.

OTHER RELATED OBSERVATIONS

It was felt that a non-statistical approach should also be made to the problem of whether carcinoma of prostate was associated with a bleeding tendency attributable to abnormal fibrinolysis. Possibly one or two isolated cases might form a group by themselves and in that sense be clinically significant but yet be too few to affect the statistical conclusion.

The lysis times of all cases in the main series falling outside the range of twice the standard errors, shown in figure 12, were placed together but no obvious clinical homogeneity was noted. Therefore a stricter standard was applied. All lysis times (P_{50}) from table XXXII and table XXXIII which are less than an arbitrary figure of 20 hours have been collected in the following table. These figures are from the main series and its controls, comprising 206 patients. Only first examinations are included.

TABLE X

The nine instances out of the main series and the controls where the 50 per cent clot lysis time was 20 hours or less

Name	Age	Diagnosis	In- or out-patient	Lysis time P_{50} (hours)	Plasma fibrinogen g./100 ml.
Case 2	83	Carcinoma of prostate	In	4	0.49
Case 8	73	Carcinoma of prostate	In	7	0.26
J.B.	71	Carcinoma of prostate	In	19	0.61
W.F.	60	Varicose Veins	In	16	0.43
H.W.	66	Carcinoma of bronchus	In	10	1.06
W.W.	76	Carcinoma of ileum	In	18	0.54
E.S.	63	Carcinoma of prostate	Out	8	0.46
J.E.	70	Carcinoma of prostate	Out	14	0.43
S.H.	72	Carcinoma of prostate	Out	20	0.44

Those patients included in table X, whether as in-patients or as out-patients, were several of them ill but none of them, with the possible exception of case 2, which will be described on page 147, was obviously bleeding or bruising on the day of the venepuncture. Thus the patients whose P_{50} lysis times are less than 20 hours do not appear to form a homogeneous group by showing evidence of a bleeding tendency, such as would be in contrast to the remaining 198 whose P_{50} lysis times were longer than 20 hours.

If one examines the in-patient lysis figures, three come from the main series of carcinoma of the prostate and three from the control series. The influence of age on fibrinolysis was more apparent in the main series, where 37 patients were over 70 years old, as compared to only 27 in the control series. The two least figures are from patients who had carcinoma of prostate. No conclusion seems possible from this approach.

The range of the three out-patient figures is similar to the range of the out-patient figures which will be presented in figure 15. There the fast lysis is attributed to a factor operating in some patients when and because they are out-patients. It is assumed, therefore, that the fast rate of lysis here is related in some way to the fact that the men were out-patients at the time of the examination.

Finally note was made of all patients whose whole plasma clot (P_{100}) had lysed by 24 hours. This is in line with the approach made by Tagnon, Whitmore and Shulman (1952).

Such lysis occurred in three out of the 55 in-patients in the carcinoma of prostate series. The first was in case 2 (see page 147) where there was purpura and some haematuria, the patient being examined because of this; the second was in case 8 (see page 165) where there was no clinical evidence of a bleeding tendency; and the third was in a man aged 58 where the diagnosis was confirmed histologically and the carcinoma had not obviously metastasised. He showed no evidence of a bleeding tendency at the time, and was well clinically two years later. In the control series there was only one patient where the whole plasma clot lysed in less than 24 hours. This was in a man aged 71 who had been readmitted one week before because of pyuria. Prostatectomy six weeks previously had shown benign prostatic hyperplasia.

There was thus no homogeneity with regard to a bleeding tendency in this group of four patients where P_{100} was less than 24 hours.

Conclusion: While statistical analysis has shown a convincing positive effect of age on fibrinolysis affecting the main series and the controls alike, as discussed in chapter three, it has failed to show the presence of abnormal fibrinolysis peculiar to carcinoma of the prostate. There was no difference between the 68 patients in the carcinoma of prostate group and 138 patients in the control group. Statistical analysis has also shown that calcium in the buffer has not affected the conclusions based on the lysis time of the 50 per cent clots.

Using a direct approach, it has not been possible to show a satisfactory correlation between fast lysis times and clinical bleeding, either in the main series or in the control series. The incidence of lysis of whole plasma clots within the first 24 hours is approximately 5 per cent in the main series, when attention is restricted to the 55 who were in-patients. This is presented here in order to supply a figure which is roughly comparable with, but only half the size of the figure of 12 per cent given by Tagnon and his colleagues (1953) in a series of 48 such patients. If the only two patients (case 1 and case 2) who were examined because of a bleeding tendency are excluded from the series at this stage because of bias of selection, the incidence of such lysis is reduced to two out of 53 in-patients (4 per cent), which is not materially different. It is felt, on the basis of evidence which has been presented, that such figures are arbitrarily derived and are without known clinical importance. Moreover, the incidence of abnormal fibrinolysis, whether it has clinical significance or not, is of little interest unless environmental influences are reduced to a minimum and unless the influence of age is taken into account when assessing results.

CASE HISTORIES WITH INDIVIDUAL COMMENTARIES

Case history 1

The history and laboratory findings of a certain patient suffering from carcinoma of the prostate played an important part in the evolution of this work. It seems correct, therefore, that the case history of this patient should be given some prominence, together with a report of the laboratory findings. Because the early part of the story has already been published (Swan, Wood and Daniel, 1957) only a summary will be presented at this stage and this will cover, in addition, the remainder of the patient's life. Greater detail of the case, much of it taken from the publication of Swan, Wood and Daniel, has been included in appendix 1. These details are of the same authorship as here, except for the early clinical case history.

A man E.S. aged 71 was admitted to hospital on 6.3.56. because of recent haematuria and a history over the previous six months of backache and urinary symptoms. A firm diagnosis of carcinoma of the prostate was made after consideration of the results of rectal examination, total serum acid phosphatase of 220 Gutman units per 100 ml. and, ultimately, the histology of the prostate itself. Before going to the operating theatre he had appeared as well as might be expected, but during the course of prostatic biopsy (using the perineal approach) and orchidectomy, the patient began to bleed locally. Very soon afterwards his blood pressure fell and severe shock developed,

associated with a generalised bruising and bleeding from all raw areas. Fears were felt for his survival. In the laboratory his blood was found to be almost without fibrinogen, using the method quoted by Scott (1955) of adding thrombin to oxalated whole blood. Treatment by intravenous fibrinogen was apparently life saving, and the patient recovered uneventfully thereafter. Investigations on specimens of blood removed at the time of the shock, and next day, confirmed the low fibrinogen level, but failed to show any fibrinolysis by two methods. Stilboestrol therapy was begun ten days afer the operation.

In the course of the following three years the patient was admitted to hospital a further four times. The reasons for his readmissions were straightforward at first. He suffered from the formation within the bladder of calculi and debris. Although he underwent multiple endo-cystoscopies and one supra-pubic cystostomy, there was no suspicion of further hypofibrinogenaemia or of the development of abnormal fibrinolysis. These were sought each time, lest there should be a recurrence of trouble. It was noted, however, on the fourth admission that the fibrinolysis times had become shorter, using identical techniques, although they were not outside the apparent normal range.

Not until his fifth and final hospital admission, about three years after the episode of acute hypofibrinogenaemia, was there clear evidence of further haematological trouble.

The cause for admission on this occasion was severe haemoptysis and was associated with ready bruising into the skin. The fibrinogen level was 0.08 g./100 ml., which was greater than that during the acute episode when it had been less than 0.02 g./100 ml. The fibrinolysis times had shortened so much as to be apparently significant, and the platelet count was clearly below normal on two occasions (see table XI).

He died shortly afterwards at home and no autopsy was performed. The clinical story of his death was suggestive of a massive pulmonary infarction as the result of an embolus which may have come from the leg. In comment, it may be said that proof of increasing vascular thrombosis in the presence of such active plasma fibrinolysis, as was shown latterly in this patient, would have been of near fundamental importance to the understanding of the significance of fast fibrinolysis in vivo. It is therefore a source of great regret that this observation was lost to us because he died at home.

The important laboratory findings on all five admissions have been summarised in table XI, and the relationship of demonstrable fibrinolysis to plasma fibrinogen levels can be seen in figure 14.

TABLE XI

The time relationship of various investigations which were repeated during five admissions to hospital (patient E.S.)

Date	Plasma fibrinogen g.%	Fibrinolysis time (hours)	Acid phosphatase G.U./100 ml.	Alkaline phosphatase K.A.U./100 ml.	Urea mg.%	Hb 100% = 14.8 g	Platelets /c.mm. (Lempert)
16.2.56.	-	-	220	30	-	-	-
FIRST ADMISSION							
8.3.56.	-	-	82	24	-	97	-
14.3.56.	<25	No lysis *	-	-	-	-	-
15.3.56.	80	No lysis *	-	-	-	72	150,000
19.3.56.	390	Similar to control *	-	-	-	68	-
20.3.56.	-	-	16	-	44	-	-
27.3.56.	-	-	12	47	-	64	-
6.4.56.	-	-	7	52	-	-	-
13.8.56.	-	-	4	14	31	-	-
SECOND ADMISSION							
17.10.56.	380	P_{50} = 96	-	-	22	108	-
THIRD ADMISSION							
26.11.56.	460	P_{50} = 99	2	-	-	-	-
FOURTH ADMISSION							
26.8.58.	280	P_{50} = 32	7	23	37	90	165,000
3.9.58.	320	P_{50} = 23	-	-	-	-	-
26.1.59.	-	-	46	34	46	-	-
FIFTH ADMISSION							
12.2.59.	80	P_{50} = 6	30	32	-	72	40,000
17.2.59.	75	P_{50} = 5	-	-	-	63	-
#19.2.59.	-	-	-	-	-	64	198,000
21.2.59.	60	-	-	-	-	58	-
24.2.59.	80	P_{50} = 8	-	-	-	62	-
26.2.59.	-	-	-	-	-	63	107,000
2.3.59.	80	P_{50} = 6	26	58	-	63	-

* method of assay described in appendix 1. # Corticotrophin injections were begun on 19.2.59.

Acid phosphatase has been measured in Gutman units (G.U.) and alkaline phosphatase has been measured in King Armstrong units (K.A.U.).

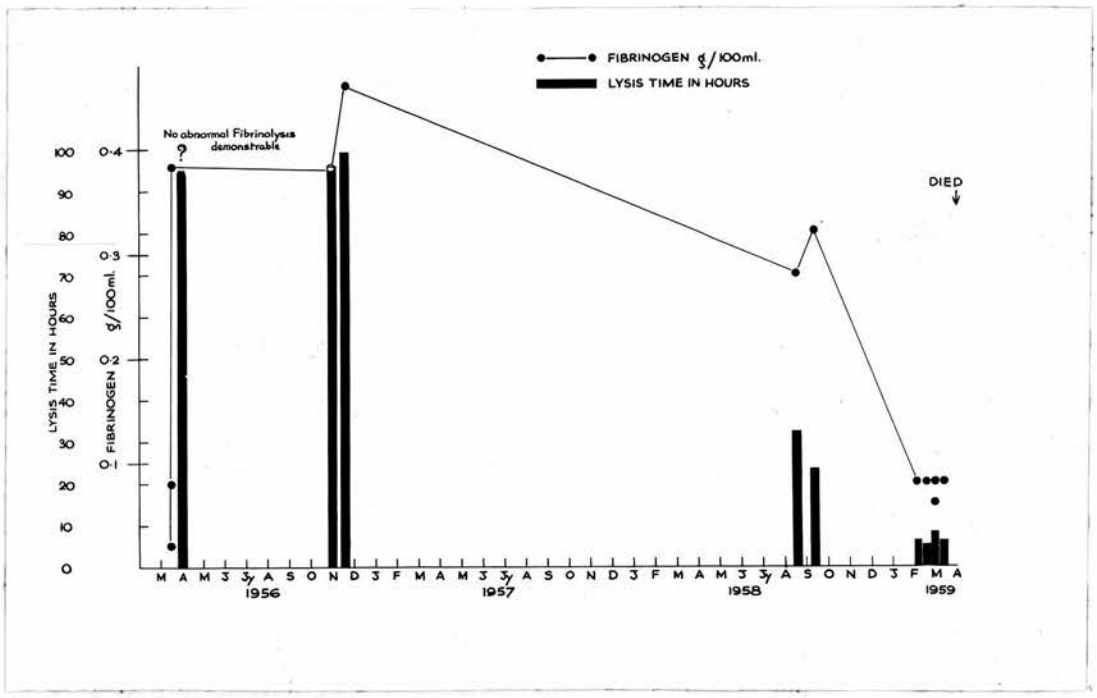


FIG. 14: Patient E.S. The relationship of assays of fibrinolysis (P_{50}) and plasma fibrinogen, plotted against time. Fibrinolysis assays in March, 1956 were made by a different technique from those made subsequently - see text.

Without venturing at this stage on an explanation of the events, it would appear that the dramatic first episode was of intense loss of fibrinogen without fibrinolysis and of very short duration. The middle two to three year period was one of urinary troubles with normal plasma fibrinogen and without any abnormal fibrinolysis. The third period was pre-terminal and was associated with a partial depression of fibrinogen and severe fibrinolysis and thrombocytopenia. Points arising from this case history are discussed from time to time in the text.

Case history 2

The case history of another patient is of sufficient importance to justify inclusion here in some detail. Laboratory observations have been included.

An 83 year old man E.A.S. was admitted as an emergency case on 6.5.56. because of acute retention of urine with over-flow incontinence. There was some haematuria. He gave a history of urinary troubles for the previous three months. On examination the prostate was enlarged and very hard, feeling like a carcinoma. The blood pressure was 190/120 mm. Hg. and there was clinical evidence of left ventricular failure.

On the day of admission he threw two fits showing all the characteristics of epileptic fits, but lacking any preceding aura. They were followed by amnesia. The fits were attributed

to cerebral arteriosclerosis, or, less likely, to hypertensive encephalopathy or a combination of both (and there was also a raised blood urea). The intention had been to remove the prostate surgically in order to relieve urinary obstruction, but his general condition was poor, and it was considered that he was temporarily unfit for surgery. He was therefore transferred to a medical unit for treatment of "left sided heart failure secondary to hypertension and myocardial ischaemia". He was given digoxin and a mercurial diuretic ("mersalyl") and from 7.5.56. was given stilboestrol 5 mg. thrice daily. He had not taken oestrogen therapy before.

Nine days later (15.5.56.) there was further haematuria and he developed purpura on the legs and trunk with some bruising. Mersalyl injections were not stopped and the purpura subsequently cleared up despite this.

As a result of the fibrinolysis investigation of 26.5.56. and 6.6.56. the dose of stilboestrol was doubled. A third assay of fibrinolytic activity on 18.6.56. was apparently normal. During the latter period the patient's general condition had improved slightly, but his complete dependence on a catheter forced the decision to operate, there being no longer a strong haematological contraindication. He was returned to a surgical ward, but on the night before the intended operation he fell out of bed and died.

Autopsy on 27.6.56. was about 36 hours after death. There was a long bruise on the chest without an associated fracture.

Most of the blood in the body was fluid. No solid clots were seen. Sternal bone marrow appeared to be in good condition. Widespread atheroma of blood vessels was found. In the brain there was an area of cavitation in the occiput, the area being rich in stainable iron. This was presumably the seat of the epileptiform attacks. In the light of the autopsy findings and after consideration of the histology, the cause of death was considered to be bilateral pyelonephritis in a patient suffering from adenocarcinoma of the prostate. The brain lesions were regarded as resulting from old infarcts: adhesions in the abdomen were attributed to healed obliterative tuberculous peritonitis. There was also left ventricular hypertrophy. There was no evidence of metastases from the adenocarcinoma.

Table XII summarises some of the investigations.

TABLE XII

Some of the haematological findings (patient E.A.S.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours		Acid phosphatase G.U./100 ml.	Urea mg.%	Hb.% 100% = 14.8 g.	Platelets/ c.mm.	Prothrombin time (control = 15")	Lee & White clotting time min.
		Whole plasma clot lysis	P_{50}						
7.5.56.	-	-	-	3.1	91	101	-	-	-
Stilboestrol 5 mg. thrice daily (from 7.5.56.)									
26.5.56.	490 *	8	4	-	-	-	250000	16	$6\frac{1}{2}$
6.6.56.	620 *	7	5	-	-	-	-	16	-
Stilboestrol 10 mg. thrice daily (from 14.6.56.)									
18.6.56.	-	46	29	-	-	80	156000	-	10
25.6.56.	DIED								

* estimated by a turbidometric method (Podmore, 1959). The Kjeldahl method was impossible to perform because the clot which had appeared normal at its formation lysed shortly afterwards and before the investigation could be completed.

Other relevant observations are as follows:-

1. The sterile whole blood clot of 26.5.56. had almost disappeared after two hours at 37°C. The whole blood clot of 6.6.56. behaved similarly.

2a. Sterile plasma of 26.5.56. was kept at 4°C for two days. It was then diluted 50 times and recalcified. The clot appeared normal and was left at room temperature, but had lysed by the following morning. When 1 ml. of normal plasma was added to the same tube, it clotted normally but this clot also had disappeared by the subsequent day, although kept only at room temperature.

2b. No thermal range of activity was demonstrated, but it was noted that the clots formed by the addition of thrombin to oxalated plasma in serial dilution 1:10 to 1:320 (as part of an emergency method of estimating the fibrinogen content in the plasma (Wolf, 1954)) all lysed within six hours at room temperature.

3. As an independent experiment some plasma obtained on 26.6.56. was diluted 1:100. One part was recalcified and the other part was clotted with thrombin, being kept at room temperature. The thrombin clot lysed more than six hours before the calcium clot.

4. Observations on the thromboplastic and fibrinolytic effect of extracts of this patient's prostate, removed at autopsy, are made in the appropriate section devoted to *in vitro* tests with prostate extracts.

Summing up the case as a whole, there appear to be certain points of importance.

Abnormal fibrinolysis was first suspected on the basis of purpura in a patient diagnosed as suffering from carcinoma of the prostate. Proof of the connection between purpura and this pathology cannot be supplied because it was seen so rarely.

Powerful fibrinolysins were demonstrated in the same specimen of plasma as also contained a normal or increased amount of fibrinogen. This implies fibrinolysis without apparent fibrinogenolysis in vivo.

The fibrinolysin survived well in its own plasma, both at room temperature and at 4°C for two days, and during that time did not appear to lyse fibrinogen in the same specimen. A good clot could subsequently be formed from such plasma but, being formed, was lysed shortly afterwards, even at room temperature. Thus we have fibrinolysis without obvious fibrinogenolysis in vitro, and the maintenance of powerful fibrinolytic activity at room temperature. This was the only patient where the Cullen and van Slyke (1920) clot lysed entirely before the assay, and the observation was therefore controlled by almost all the remaining fibrinogen assays.

This patient's fibrinolysin was active at room temperature against the clot from a normal person.

Considerable diminution of fibrinolysis and some clinical improvement coincided with the doubling of the stilboestrol dose to 30 mg. a day. It is impossible to be certain that improvement in fibrinolysis was unrelated to the treatment which he was receiving for heart failure.

This is an example of increased fibrinolysis, possibly of a pathological type, in a patient suffering from histologically proven carcinoma of the prostate without demonstrable metastases.

Case history 3

A 66 year old man F.V. was admitted into a surgical ward from 5.5.56. until 21.5.56. He was suffering from chronic ill-health, loss of weight and backache. He was found to have a hard enlarged prostate, bony destruction of the bodies of lumbar vertebrae 1, 2 and 3, consistent with metastases, and his serum acid phosphatase level was raised as shown in table XIII. After a period of grave illness he improved dramatically within a day or two of starting stilboestrol therapy, but survived only eleven months. He did not die in hospital.

TABLE XIII

The more important laboratory findings (patient F.V.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours P_{100}	Fibrinolysis in hours P_{50}	Acid phosphatase G.U./100 ml.	Alkaline phosphatase K.A.U./100 ml.	Urea mg.%	Hb.% 100% = 14.8 g.	Platelets/c.mm.	Serum calcium mg.%
8.5.56.	0.40 *	++	47	24	9	-	54	180000	14.1
	Prostatic massage followed by repeat test half an hour later.								
	-	++	46	-	-	-	-	-	-
	Stilboestrol 30 mg. thrice daily then begun.								
11.5.56.	-	-	-	28	11	-	-	-	14.3
15.5.56.	-	42	21	22	-	-	50	-	-
18.5.56. (O.P.)	-	-	-	7	22	68	-	-	-
16.6.56. (O.P.)	-	-	-	1	70	19	41	-	9.5
2.7.56. (O.P.)	0.35	<17	<17	-	-	-	50	240000	-
16.7.56. (O.P.)	-	<16	<16	-	-	-	52	200000	-
	Stilboestrol 10 mg. thrice daily from 26.11.56.								
18.2.57.	-	-	-	23	-	-	81	-	-
4.3.57.	Deteriorating.				April 1957 DIED				

O.P. = out-patient examination * estimated by Wolf (1954) method only.

Points of note:

1. Although bony metastases were apparent when he was first seen, no abnormal fibrinolysis was demonstrated in his plasma.
2. During a period when he was beginning to feel very much better and appeared to be responding clinically to stilboestrol, the lysis time did not lengthen. If anything, it shortened (15.5.56.)
3. Prostatic massage did not shorten the lysis time. This is discussed in the section devoted to prostatic massage, later in this chapter.
4. The two out-patient assays of fibrinolysis were very much faster than earlier ones, and at these times the patient was feeling very well. Although the omission of night observations made the P_{50} readings unsatisfactory on 2.7.56. and 16.7.56. (the lysis time was presumably considerably less than 17 hours), the whole plasma clot lysis by 17 hours was nevertheless significantly fast.
5. This patient's plasma was used for a laboratory experiment concerning potency after storage and adsorption, described later.

Case history 4

A 64 year old man T.L. was referred to hospital because of pain in the anterior urethra, felt on micturition, and a pain in the left lumbar region, relieved by micturition. The prostate was big and hard.

1st admission: 21.9.56. - 27.9.56. Cystoscope was passed with difficulty through an obviously malignant prostate. The bladder was dirty and intramural extension of malignant growth from the prostate was considered possible. He was given stilboestrol 90 mg. a day.

2nd admission: 20.11.56. - 23.11.56. Cystoscopy. Bladder mucosa cleaner.

3rd admission: 30.1.58. - 5.2.58. General and local deterioration. Buttocks feeling frozen. Bilateral orchidectomy performed. This operation was followed by symptomatic improvement for a while. Less pain. Appetite better.

4th admission: 10.7.58. Acute retention of urine for 24 hours. He developed minor fits. Increasing urinary failure until he died four days later.

Autopsy: Carcinoma of prostate with secondaries in bladder, lymph nodes and liver (none in lumbar vertebral bodies). Bilateral hydroureter and hydronephrosis.

TABLE XIV

The more important laboratory findings (patient T.L.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours		Acid phosphatase G.U./100 ml.	Alkaline phosphatase K.A.U./100 ml.	Urea mg.%	Hb.% 100% = 14.8 g.	Platelets /c.mm. (Lempert)
		P_{100}	P_{50}					
24.9.56.	0.48	70	35	1	-	-	100	210,000
Stilboestrol 30 mg. thrice daily (from 25.9.56.)								
31.1.58.	0.34	71	55	2	11	-	-	-
	Bilateral orchidectomy followed in 15 minutes (7 p.m.) by							
	0.36	16	14*	-	-	-	-	-
17.2.58.	0.25	35	23	3	7	-	-	-
10.7.58.	-	-	-	-	-	202	-	-
14.7.58.	DIED							

* this figure of 14 hours is almost certainly in excess of the true reading, due to failure to read the tubes through the night.

Points of note:

1. Considerable increase of fibrinolysis was found 15 minutes after surgery (bilateral orchidectomy under general anaesthesia). The fibrinogen level remained constant.
2. No convincing evidence of any speeding up of fibrinolysis during the last two years of the patient's life.

Case history 5

A 69 year old man F.P. had been diagnosed in 1953 as suffering from carcinoma of the prostate. This had been discovered almost incidentally in the course of follow-up of bladder papillomatosis by which he had been troubled intermittently since 1943. A biopsy of the prostate was reported as showing a spheroidal cell adenocarcinoma. He was then treated with stilboestrol for two years. Just before this admission he lost control over micturition. He was admitted to hospital on 28.7.57. with a view to relief of urinary obstruction. Orchidectomy and trans-urethral resection of the prostate were performed. Stilboestrol was

resumed after having been stopped for over a year. X-ray of lungs, skull, pelvis and spine showed no evidence of metastases. Despite treatment his general condition deteriorated. He died uraemic, in hospital on 8.2.58. Autopsy showed carcinoma of the prostate and bilateral hydronephrosis with hydroureter.

TABLE XV

The more important laboratory findings (patient F.P.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours P_{100}	Fibrinolysis in hours P_{50}	Acid phosphatase G.U./100 ml.	Alkaline phosphatase K.A.U./100 ml.	Urea mg.%	Hb.% 100% = 14.8 g.
2.1.58.	0.66	98	50	3	11	-	86
Bilateral orchidectomy 3.1.58.							
13.1.58.	0.69	103	103	-	-	-	72
22.1.58. (4 p.m.) Trans-urethral resection of the prostate completed							
(4.30 p.m.)	-	16	15	-	-	-	78
28.1.58.	0.80	74	79	-	-	36	67
1.2.58.	-	-	-	-	-	56	-
Stilboestrol 10 mg. thrice daily from 2.2.58.							
4.2.58.	-	-	-	-	-	84	-
6.2.58.	0.90	47	20	-	-	126	-
8.2.58. DIED							

Points of note:

1. Active fibrinolysis of the immediate post-operative period was demonstrated.
2. There was no clear-cut evidence of the effect of bilateral orchidectomy on fibrinolysis.
3. The plasma fibrinogen level increased as the illness progressed.

Case history 6

An 81 year old man W.C. was admitted to hospital on 28.8.58. because of acute retention of urine over the previous four days. He had suffered from urinary trouble ten years previously, for which prostatectomy had been performed. It was not possible to find out the nature of the tissue removed but it was presumed to have been benign. On this occasion malignancy was not suspected until the operation of trans-vesical excision of the left lobe of the prostate and resection of the bladder neck, when a craggy mass was found with apparent infiltration at the site of the former resection. The first specimen for fibrinolysis assay was taken in the post-operative period, about four hours after the patient returned from theatre and before any stilboestrol had been given. No evidence of metastases was found at any time.

TABLE XVI

The important haematological features (patient W.C.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours		Whole blood clot at 24 hours	Acid phosphatase G.U./100 ml.	Urea mg.%
		P_{100}	P_{50}			
11. 9.58.	0.58	46	48	Clot	-	56
Stilboestrol 10 mg. daily from 13.9.58. 10 mg. thrice daily from 23.9.58.						
25. 9.58.	0.64	23	23	Lysed	15	-
10.10.58.	-	23	22	Lysed	-	-
11.10.58.	0.44	22	22	Lysed	-	-

Points of note:

1. There was no obvious increase of fibrinolysis four hours after operative surgery.
2. Three subsequent assays of fibrinolysis over a span of 17 days are consistent within themselves. It is unusual to find the whole blood clot lysed by 24 hours and this is often accepted as clear evidence of increased fibrinolysis, but in the light of work presented here the advanced age of the patient should be taken into consideration.

Case history 7

A 68 year old man W.J. was admitted to an orthopaedic ward on 25.6.59. complaining of severe pain in the back but no urinary symptoms. He gave a history of feeling an ache around the shoulders starting about five months previously. By the time of his admission the symptoms were consistent with the radiological appearance of rarefaction of the first and second lumbar vertebrae with a probable compression fracture of the latter and a possible secondary deposit in the third and fourth cervical vertebrae. The diagnosis of carcinoma of the prostate was made on rectal examination and on the strength of an unusually high serum acid phosphatase level. He was treated by traction and with stilboestrol. His pain was not relieved easily and morphine was necessary at first. When last seen in November 1960 he was greatly improved and could walk without sticks.

TABLE XVII

The important haematological findings (patient W.J.)

Date	Plasma fibrinogen g.%	Fibrinolysis in hours		Acid phosphatase G.U./100 ml.	Alkaline phosphatase K.A.U./100 ml.
		P_{100}	P_{50}		
7. 7.59.	0.48	47	56	860	24
Stilboestrol 5 mg. thrice daily (from 8.7.59.)					
14. 7.59.	0.77	71	30	-	-
21. 7.59.	-	79	55	-	-
Stilboestrol 5 mg. daily					
24. 8.59. (O.P.)	0.46	31	23	35	84
2.11.59. (O.P.)	-	-	-	18	66
1.12.59. (O.P.)	0.41	45	23	11	8
11. 4.60.	-	-	-	2	-

O.P. = out-patient examination

Points of note:

1. There seems to be no apparent relationship here between very high serum acid phosphatase levels and fibrinolysis. The observation on 7.7.59. that the whole plasma clot lysed before the 50 per cent clot is not unique and need bear no relationship to this.
2. Both fibrinolysis assays which were made when the patient was an out-patient are faster than those made as an in-patient. The significance of this is discussed elsewhere.

Case history 8

A 73 year old man J.D. was referred to the ear, nose and throat department where he was admitted on 28.11.57. Two days previously he had developed sudden dysphagia. Until that time he had swallowed quite normally, but suddenly found that he could take neither solids nor adequate fluids, although he managed to swallow very small amounts of liquid and to suck lozenges. His previous history included the operation of excision of the bladder trigone and wedge resection of the left lobe of the prostate on 17.1.57. Histological examination of the prostate showed an extensive poorly differentiated small alveolar-cell carcinoma. Metastases were not recognised. He had taken stilboestrol tablets to a total daily dose of 20 mg. until the onset of the dysphagia.

The pharynx and larynx were examined clinically but nothing was seen to account for acute dysphagia. The pharynx was red and oedematous. A barium swallow showed no abnormality about a week after the onset, by which time his swallowing was improving. Cervical osteophytes were seen which "could account for the symptoms". The first and only fibrinolysis assay was made on the day of his discharge from hospital, although he was still in bed at the time the specimen was removed. The assay showed unusually fast lysis, all clots, including the whole blood clot, having lysed by eight hours. The patient was not seen again and is believed to have died within the subsequent three months.

Comment: Complete lysis by eight hours was one of the fastest rates of lysis demonstrated in any patient. It is put forward as a suggestion that the patient had bled into his paraoesophageal tissues, and so had developed dysphagia, but no proof for this can be offered. The fact that the patient was due to go home that day could have caused excitement in the patient and thus led to a falsely fast rate of lysis, although it is recalled that the man showed no outward evidence of excitement at the prospect of going home.

The remaining 60 case histories have not been presented individually here because of the sameness of their story and because of the lack of individual distinction about most of the results obtained from them. The clot lysis times of all patients, together with the corresponding plasma fibrinogen level, have been listed in table XXXIII in appendix 2. An indication has also been given there of cases where the diagnosis was proved histologically, and those where the diagnosis was beyond reasonable doubt, as already described. Although no further formal case histories will be presented, relevant information concerning the clinical course of certain patients will be supplied in appropriate sections.

The remainder of this chapter will be devoted to observations made on the data with a view to clarifying uncertainties surrounding the influence of metastatic spread, oestrogen therapy and bilateral orchidectomy on fibrinolysis associated with carcinoma of the prostate. The effect on fibrinolysis of massage of a prostate known to be the seat of malignant disease will also be described. In addition to supplying observations on two physical properties of an actively fibrinolytic plasma, laboratory experiments have been carried out into the thromboplastic and fibrinolytic properties of prostatic tissue. These will be presented.

THE PART PLAYED BY METASTASES IN DETERMINING THE RATE OF PLASMA FIBRINOLYSIS.

Metastases were recognised in 22 out of the 68 cases of carcinoma of the prostate (see table XXXIII in appendix 2). 16 of these were in-patients at the time of the examination. When the P_{50} lysis times of the in-patients were compared to the mean for the appropriate age in the carcinoma of prostate group, as is shown in figure 12, the points all fell equally round the mean. Only one of them (P_{50} = 19) was clearly less than twice the standard error below the mean. This patient, J.B., a man of 71 years, was in considerable discomfort at the time of the examination, due, it was believed, to secondary osteoplastic deposits which were present in the upper lumbar spine, with possible nerve root involvement. The diagnosis of carcinoma of the prostate was made on clinical and radiological grounds, without histological evidence. There was no evidence of a bleeding tendency at any stage of his illness. This patient thus showed no unusually distinctive qualities such as would put him in a class apart from the others, except by virtue of a slightly faster rate of fibrinolysis.

It is considered of great interest in this context that unusually vigorous lysis which was demonstrated on two occasions on the plasma from E.A.S. (case 2) was found in a patient on whom subsequent autopsy showed no metastases.

Comment: Patients known to have metastases from prostatic carcinoma do not appear to be any more likely to demonstrate unusually fast rates of plasma lysis than the group as a whole.

THE INFLUENCE OF OESTROGEN ON FIBRINOLYTIC ACTIVITY.

Ten patients who had prostatic carcinoma were examined before receiving stilboestrol therapy and again while receiving stilboestrol. Three of these ten patients were examined only once in the ward and once in the out-patient department. As a result of the uncertain validity of comparisons made between in-patient and out-patient figures (this will be discussed later), these three patients have not been included in the following table. Those who remain were in-patients at the time of both examinations. If multiple examinations were made, the last before stilboestrol therapy and the first during such therapy were selected. The dosage employed varied from 10 - 90 mg. a day, most commonly 15 or 30 mg. in divided doses. Table XVIII shows the differences which were found.

TABLE XVIII

The possible effect of oestrogen on fibrinolysis.

Name	Lysis times in hours		Time interval between assays (months)	Increase (+) or diminution (-) of lysis
	Pre-oestrogen therapy P_{100}: P_{50}: P_{10}	During oestrogen therapy P_{100}: P_{50}: P_{10}		
P.A.	96: 59: 48	++: ++: 77	16	-
H.W.	48: 41: 31	29: 22: 5	1	+
W.C.	46: 48: 57	42: 23: 19	4	+
C.C.	++: 98: ++	++: 62: 24	36	+
W.J.	47: 56: 28	71: 30: 23	1	?+
T.L.	70: 35: 22	71: 55: 25	64	-
F.V.	++: 47: 42	42: 21: 22	1	+

++ no lysis by 240 hours

Comment: There is probably no important significance in these figures unless to cast great doubt on the ability of oestrogen to diminish fibrinolysis as assayed in these patients. It is possible to cite the example of E.A.S. (case 2), who was already receiving stilboestrol and therefore is not included in this table, as one where the patient improved clinically when the stilboestrol dose was doubled to 30 mg. daily and whose lysis rate diminished simultaneously. It is equally possible to cite the case of F.V. above, to support the opposite point of view. This patient suffered from metastatic carcinoma of the prostate with destruction of vertebrae and had a brick hard enlargement of the prostate with a raised serum acid phosphatase level. He improved "dramatically" on stilboestrol therapy, but his plasma fibrinolytic activity four days after starting oestrogen therapy, when he was already feeling much better was, if anything, greater than it had been before.

Clearly there is still a place for studying the influence of oestrogen on "pathological" fibrinolysis in carcinoma of the prostate, assuming that such exists as an independent entity. Except for a rare case, however, it is difficult to know just what constitutes pathological fibrinolysis specifically associated with carcinoma of the prostate. E.A.S. (case 2) is the best example in this series of apparently pathological fibrinolysis, but his advanced age of 83 is just

the age when fibrinolysis appeared most active in the control series. Too few assays were made before the patient died to allow one to be certain of even this one case as a reliable example.

The possibility that oestrogen may increase the rate of lysis in certain patients should be borne in mind as a result of table XVIII where the lytic activity appears a little more pronounced in five out of seven patients after starting oestrogen therapy. Patients suffering from carcinoma of the prostate are not appropriate for studying the influence of oestrogen on physiological fibrinolysis because of the undesirable number of variables and unknowns which are present.

THE EFFECT ON FIBRINOLYSIS OF ORCHIDECTOMY FOR PROSTATIC CARCINOMA.

Two patients suffering from carcinoma of the prostate were deemed to require bilateral orchidectomy. Plasma was examined before and after the operation. Table XIX shows the result.

TABLE XIX

Fibrinolysis before and after orchidectomy.

Name	Age	Lysis times in hours		Increase (+) or diminution (-) of lysis
		Before operation P_{100}: P_{50}: P_{10}	After operation P_{100}: P_{50}: P_{10}	
F.P.	69	50: 50: 25	++: 103: 71 (10 days after operation)	-
T.L.	64	71: 55: 25	79: 23: 11 (17 days after operation)	+

++ no lysis by 240 hours

The rate of fibrinolysis was not obviously abnormal in either of these patients before orchidectomy, and there is nothing in the post-operative rates of lysis to suggest an important change in them at the times of the examinations.

PROSTATIC MASSAGE

Effect on plasma fibrinolysis and fibrinogen

Shortly after a patient (case 1) had almost died as a result of hypofibrinogenaemia in the course of prostatic biopsy, it was felt that the precipitating cause could have been the release of tissue substance from the cancerous prostate. It was therefore decided to massage the prostate of patient F.V. (case 3), who was known to be suffering from carcinoma of the prostate, and watch the effect. The risk involved was thought to be small because of the close observation being made. The prostate was massaged for five minutes. At the end of a further twenty five minutes a blood sample was taken and a ten-tube fibrinolysis test set up. The results of this test were compared with an identical test which had been set up immediately before the massage began. The lysis time of the 50 per cent plasma clot (P_{50}) was 47 hours as measured before massage and 46 hours as measured after massage (table XIII).

Comment: Prostatic massage had no effect on plasma fibrinolysis as assayed in this patient.

With reference to patient E.S. (case 1) who developed a fulminating hypofibrinogenaemia during a prostatic biopsy, which probably involved some prostatic massage, it should be noted that abnormal fibrinolysis was not demonstrated in him, either at the time or for two years subsequently.

Effect on serum acid phosphatase

Seven out of 68 patients diagnosed as having a

carcinoma of the prostate showed acid phosphatase levels very slightly higher than four Gutman units per 100 ml., which is the upper limit of normal for the laboratory concerned. There is no finality about four units being the exact limit, and indeed slightly higher figures are commonly accepted as being of very doubtful significance if not actually normal. These seven patients are listed in table XX below and comprise all those who had levels between four and ten Gutman units per 100 ml., provided that this was not a recession from a higher level. An indication of the timing of the specimen is also given.

TABLE XX

The timing of specimens where the serum acid phosphatase level was only slightly raised.

Patient	Gutman units per 100 ml.	Day of specimen
C.W.	7.0	At out-patient visit (rectal examination made)
J.M.	6.0	Third day after admission
E.T.	4.5	Day of cystoscopy
C.C.	4.6	No comment
D.P.	8.0	Day of cystoscopy
H.R.	4.9	First day seen (rectal examination made)
G.W.	6.0	Day after admission (? rectal examination)

No conclusion is possible from these figures and the comments made with them. The case records do not supply the time of day when the specimens were taken nor the time of all the clinical examinations, so it is impossible to relate the two retrospectively. It is suggestive, however, on circumstantial evidence, that in several of them some form of prostatic massage, either digitally or by cystoscope, was given before the blood sample was taken for acid phosphatase estimation.

This evidence, such as it is, may be relevant in the light of the observations of Daniel and van Zyl (1952) who found a rise of serum acid phosphatase level following palpation of the prostate in three out of 24 patients with benign prostatic hypertrophy.

More relevant to the work of Daniel and van Zyl is the acid phosphatase level on a patient W.W. who was feared to be suffering from carcinoma of the prostate when first admitted to hospital. The prostate was a little enlarged. There was slight difficulty with micturition, and diarrhoea. He was found to have a serum acid phosphatase level of six Gutman units per 100 ml. This specimen was known to have been taken almost immediately after rectal examination. His symptoms were subsequently shown to be due to an intestinal carcinoma and not to a carcinoma of the prostate. Later estimations of acid phosphatase were all less than four Gutman units per 100 ml.

Comment: Although prostatic massage is known to increase the output into the serum of acid phosphatases this is not peculiar to patients suffering from carcinoma of the prostate, and can occur in cases of benign hyperplasia of the prostate as in the case cited here.

TWO PHYSICAL PROPERTIES POSSESSED BY AN ACTIVELY FIBRINOLYTIC PLASMA.

1. Survival of fibrinolysin in the cold.

Plasma was withdrawn from a patient (case 3) with a known diagnosis of carcinoma of the prostate, and whose lysis time was fast (P_{100} = <17) on the occasion of an out-patient examination. A ten-tube dilution test for fibrinolysis was set up at the time, the remainder of the specimen being oxalated (Heller and Paul (1933-34) mixture), and then stored for 19 hours at 4°C. At the end of this time a second ten-tube fibrinolysis test was set up, using thrombin as clotting agent. The fresh-specimen clots were all lysed by 17 hours. The stored-specimen clots had none of them begun to lyse by six hours but had all lysed by 22 hours. (This served as a control for the adsorption experiment described below) While the two figures are not directly comparable, they are both fast, and there cannot be an important difference between the lysis times of the fresh and the stored specimen.

The survival in the cold of the fibrinolysin from case 2 has already been described.

2. Adsorption of fibrinolysin.

Four drops of prepared aluminium hydroxide (Biggs and Macfarlane, 1957a) were added to 4 ml. of the stored specimen of plasma described above. This was incubated at 37°C for 5 minutes and the adsorbed plasma separated off, after centrifuging for 5 minutes at 3,000 r.p.m. The adsorptive power of the alumina was controlled by the Quick one-stage "prothrombin" time which was prolonged on the specimen to nearly 4 minutes. A ten-tube fibrinolysis test was then set up on the adsorbed specimen. All tubes lysed between 6 and 22 hours, and in this respect did not differ at all from the unadsorbed plasma.

Comment: The active fibrinolysin in this plasma did not obviously lose its potency after storage for 19 hours at 4°C. The fibrinolysin was not adsorbed by aluminium hydroxide in a controlled experiment.

LABORATORY EXPERIMENTS ON PROSTATIC TISSUE

Thromboplastic activity of tissue extracts

Benign hypertrophy of the prostate (preparation 1, acetone-extracted)

A prostate which was shown histologically to be the seat of benign hypertrophy was removed at operation. It was washed in saline and blood vessels and membrane removed so far as this was possible with such a tough material. The

remainder was chopped finely and ground with acetone in several changes, according to the method advocated by Biggs and Macfarlane (1957b) for the preparation of human brain "thromboplastin". The residual particles were dried overnight in a dessicator. Finally, 0.5 g. of this preparation was added to 10 ml. saline. This was placed in a water-bath at 37°C for 15 minutes with inversion at intervals. The murky supernatant was then used as a potential source of thromboplastin, being run in parallel with human brain thromboplastin as a component of Quick's one-stage "prothrombin" time. The following are the results on plasma from two healthy people and from five patients on anticoagulant therapy with phenindione. Results were made in duplicate and the averages are presented in table XXI.

TABLE XXI

A comparison of thromboplastic activity in acetone-extracted prostate (benign hypertrophy) with brain similarly treated.

Source of plasma	Brain suspension	Prostatic suspension
Normal 1	13	196
Normal 2	13	65
O'B.	21.5	137
H.	33	99
B.	16	70
G.	27	153
P.	17	72

unit = seconds

Comment: There appears to be practically no thromboplastic activity in this prostate, which was the seat of benign hyperplasia, after acetone extraction.

Benign hypertrophy of the prostate (preparation 2, buffer-extracted)

A prostate which was removed at operation from another patient, aged 81, because of benign hypertrophy, was taken immediately to the laboratory. The tissue was washed in saline to remove obvious blood and was then cut with scissors and a scalpel into as fine particles as possible. The small bits were dropped into a mortar with about 8 ml. of veronal buffer at pH 7.4. The fibrous particles were ground as much as was possible, for the remainder of half an hour. The whole was then sieved crudely and the liquid part used for the following three experiments.

(a) The technique as for Quick's one-stage "prothrombin" time was performed using prostatic extract as the source of thromboplastin, and this was compared with the activity of brain thromboplastin. The effect of storing the prostatic extract at 4°C was noted. The results were made in triplicate and the means are presented in table XXII.

TABLE XXII

A comparison of the thromboplastic activity in buffer-extracted prostate (benign hypertrophy) with brain.

	Brain suspension	Prostate extract	Prostate extract 1:1 with saline	Prostate extract stored 2 days at 4°C	Prostate extract stored 5 days at 4°C	Saline
Normal plasma	14	17.5	19	18	26	99.5
Normal plasma *	16	15	-	-	-	91

unit = seconds

* plasma incubated for 30 minutes before the test with (a) brain or (b) prostate extract or (c) saline.

(b) The heat stability of the thromboplastic activity of the same preparation of prostate extract is shown in table XXIII. The figures which have been presented are the means of results in triplicate and represent the clotting times of equal volumes of normal plasma, brain or prostate extract and calcium solution.

TABLE XXIII

The effect of heat on the thromboplastic activity of prostate extract.

	Without heating	After 56°C for 10 minutes
Brain suspension	14	21
Prostate suspension	16	22

unit = seconds

(c) The same prostatic extract was filtered through Whatman filter paper No. 1, the filtrate and the residuum then being examined separately for thromboplastic activity, as already described above. The means of results in triplicate have been recorded in table XXIV.

TABLE XXIV

The different thromboplastic activities of the filtrate and residuum of prostate extract

	Brain suspension	Prostate extract (unfiltered)	Prostate extract filtrate	Prostate extract residuum
Normal plasma	14	16	19.5	16

unit = seconds

Comment: In contrast to the results of acetone extraction (table XXI), there appears to be considerable thromboplastic activity in the prostatic tissue which was prepared by veronal buffer extraction from a prostate which was the seat of benign hyperplasia. This activity is quite comparable in potency with that of brain thromboplastin (table XXII). Its activity diminished gradually with storage as a suspension over several days at 4°C. The activity seemed to reside chiefly in the particles (table XXIV). The preparation was slightly sensitive to heat at 56°C in exactly the same degree as the brain thromboplastin tested (table XXIII).

Carcinoma of the prostate (preparation 3, buffer-extracted)

At autopsy, about 12 grams of believed carcinoma tissue was selected and taken from the prostate of E.A.S. (case 2). The autopsy was made 36 hours after death. The diagnosis of carcinoma was confirmed by histological examination. This tissue was cut up and ground in 15-20 ml. veronal buffer at pH 7.4. Some of the resultant fluid was filtered through Whatman No. 1 filter paper and the remainder was pipetted off for use.

The thromboplastic activity of the preparation was assessed by Quick's one-stage "prothrombin" time. The means of results in triplicate are presented in table XXV.

TABLE XXV

A comparison of the thromboplastic activity of buffer-extracted prostate (malignant) with brain, and the effect of storage.

	Brain suspension	Prostate extract
On day of preparation	14	18
5 days later (stored at 4°C)	14 *	26

unit = seconds

* the brain suspension was freshly prepared

Carcinoma of the prostate (preparation 4, buffer-extracted)

At autopsy on a patient K.G. three days after death, that part of the prostatic tissue which looked malignant was cut out and treated similarly to the previous specimen (preparation 3) above. The diagnosis of carcinoma of the prostate was confirmed histologically. Only 5 ml. of buffer was used to prepare the extract. The resultant fluid was almost opaque even after centrifuging at 3,000 r.p.m. for 15 minutes. The supernatant fluid was used for the experiment.

The thromboplastic activity of the preparation was assessed by Quick's one-stage "prothrombin" time. When the prostatic extract was substituted for brain suspension (the control time for which was 14 seconds), the mean time was 28 seconds.

Comment: These experiments using malignant prostatic tissue from two different patients show definite evidence of their possessing thromboplastic activity. Their weaker activities compared to brain should be noted in the context of the time after death when the extracts were prepared. When preparing brain thromboplastin, it is customary to insist on as fresh tissue as possible, in order to make an efficient preparation.

Fibrinolytic activity of tissue extracts

Benign hypertrophy of the prostate (using preparation 2)

The same prostatic extract which had shown potent thromboplastic activity was mixed with normal plasma of diminishing concentrations in veronal buffer at pH 7.4, before clotting with thrombin. A range of ten dilutions of plasma was made, only the two extremes of which are recorded in table XXVI below.

TABLE XXVI

The fibrinolytic activity of (non-malignant) prostate extract.

Normal plasma	1.0	0.1	1.0	0.1
Prostate extract	0.4	0.4	-	-
Buffer pH 7.4	-	0.9	0.4	1.3
Thrombin 50 units/ml.	0.1	0.1	0.1	0.1
At 24 hours	Lysed	Lysed	No Lysis	No Lysis

unit = ml.

The experiment was repeated after storage of the prostate extract for four days at 4°C. The whole plasma clot again lysed within 24 hours, the control clot remaining intact. The 10 per cent clot was only partially lysed.

Comment: This tissue extract, from a prostate which was the seat of benign hyperplasia, was actively fibrinolytic and remained so after storage in the cold for four days.

Carcinoma of the prostate (preparations 3 and 4)

Experiments identical in method to the one just described were performed using preparation 3 and preparation 4 from prostates which were the seat of carcinoma. The results have been incorporated into table XXVII.

TABLE XXVII

The fibrinolytic activities of extracts of two carcinomatous prostates

Normal plasma	1.0	0.1	1.0	0.1
Prostate extract	0.4	0.4	-	-
Buffer pH 7.4	-	0.9	0.4	1.3
Thrombin 50 units/ml.	0.1	0.1	0.1	0.1
At 2 hours				
Preparation 3	Clot	Clot	Clot	Clot
Preparation 4	Clot	Lysed	Clot	Clot
At 24 hours				
Preparation 3	Lysed	Lysed	Clot	Clot
Preparation 4	Lysed	Lysed	Clot	Clot

Comment: The carcinomatous prostatic tissue from both these autopsies appears to have had strong fibrinolytic activity. It is not possible to exclude the possibility of contamination with organisms, but bacterial growth is unlikely to be responsible for the lysis which took place within the first two hours (preparation 4).

Summing up: The importance of these laboratory observations on tissue extracts from benign hyperplasia of the prostate and from carcinoma of the prostate is the apparent identity of their behaviour in these particular experiments. Each extract showed both thromboplastic and fibrinolytic activity.

CHAPTER FIVE

FOLLOW-UP STUDIES

Follow-up of plasma fibrinolysis levels in patients suffering from carcinoma of the prostate.

INTRODUCTION

Certain difficulties presented themselves in following patients suffering from carcinoma of the prostate when the follow-up was for purposes of research only. Almost all the patients were elderly, some of them over eighty years old, and even a visit to hospital by ambulance was a formidable undertaking. One such patient was so sick in the ambulance that he refused ever again to attend his proper surgical advisers at the hospital. In order to minimise such trouble, follow-up visits were not maintained at regular intervals. It was decided instead to note the effect of time on repeated assays made on patients already in the ward or attending the out-patient department in any case. A few patients were asked to attend for the purpose.

It was found that 20 patients had been re-examined after intervals of two weeks to three and a half years, and a comparison has been made of the figures obtained. One grouping of these figures shows a significant trend and such other information as has been gathered will be presented in relation to this.

It was decided in addition to study survival in its relationship to fibrinolysis, 34 of the 68 patients having died by the time the series was completed. Of the other 34 patients, several were untraced and may have died.

THE EFFECT OF BEING AN OUT-PATIENT

The first grouping suggested itself in the analysis from observation of the figures in relation to the clinical data. All patients who were ever examined as in-patients and subsequently as out-patients have been included in this group, without exception. There are seven such patients. They do not, of course, represent a cross section of all patients in the main series, because only the relatively fit were able to attend. However, no stock was taken of their previous fibrinolytic activity in determining their later attendance. In three of the patients the carcinoma appeared to have metastasised.

Results: Figure 15 shows the findings on these seven patients. The left half of the figure represents whole plasma clot lysis and the right half the 50 per cent clot lysis. These are two dilutions taken from the ten dilution assay and are shown together to demonstrate that the same trend is apparent in each. Each half of the figure therefore contains one line from the same experiment. The left hand point on each line represents an in-patient lysis time and the right hand point the follow-up lysis time on the same man as an out-patient. Where such a patient had more than the minimum two investigations required by this group, either in the wards or as an out-patient, the adjacent in-patient and out-patient examinations respectively have invariably been selected without prior regard to the time interval between them. The horizontal line represents time but has no scale, the time intervals varying from two weeks to 41 months, with a mean of 13 months and a median time of three and a half months.

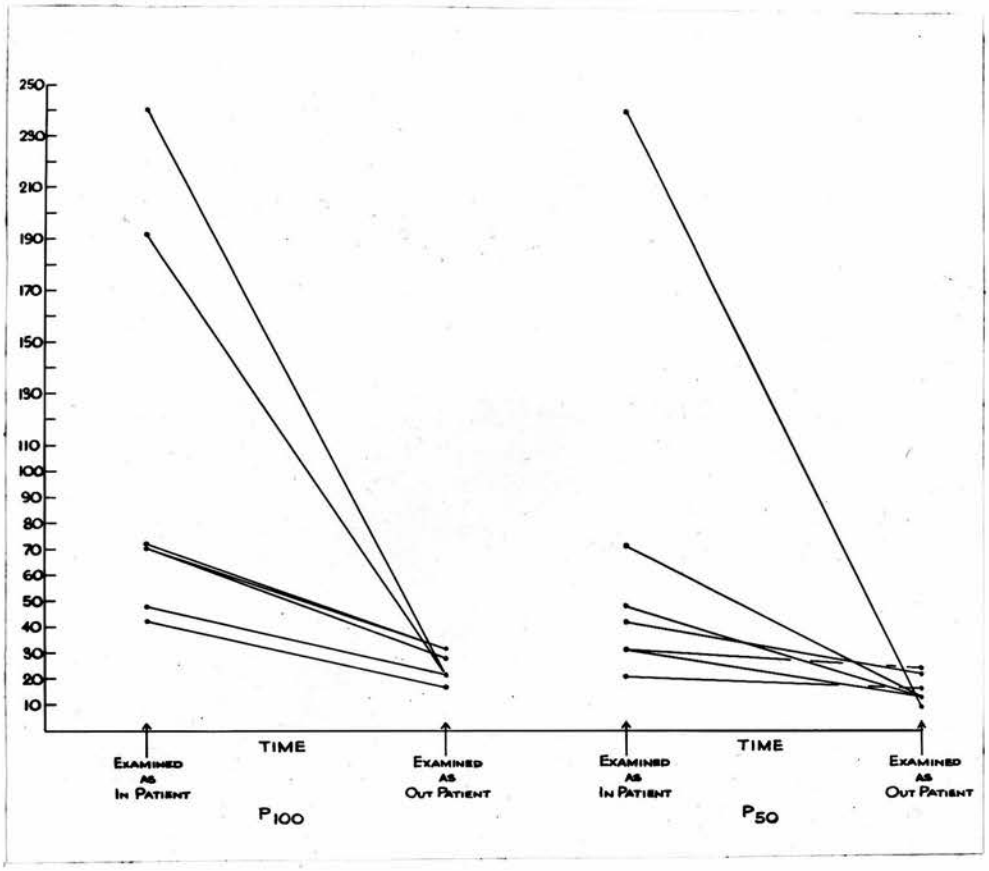


FIG. 15: The plasma lysis times of seven patients, first as in-patients and then as out-patients. The vertical scale represents the lysis time in hours.

It can be seen that the lysis time in all cases was shorter at the follow-up than it had been when last examined in the ward.

Discussion: The shortening of the lysis times at follow-up, after intervals of as little as two weeks and as long as 41 months, is obvious and uniform in the group. In explanation of this, the first suspicion should probably fall on the environmental state of the patient and upon the difference between resting in the ward and sitting in the out-patient department after a journey. On the other hand there are important alternative explanations which are related to the underlying pathology. Indeed, it was our interest in the possible increasing influence of carcinoma of the prostate upon fibrinolysis which prompted the follow-up examination in most cases. It is necessary, therefore, to discuss some of the alternative suggestions which would explain an increase of fibrinolysis with the passage of time.

THE EFFECT OF ADVANCING YEARS

The increase of lysis with advancing years on follow-up figures can certainly be discounted as an explanation of the trend shown in figure 15, where lysis has speeded up in periods as short as two weeks. A study of the composite line in figure 7 (see chapter 3) shows that the increase in lysis time with age is relatively slight, except when measured over decades.

THE EFFECT OF ADVANCING DISEASE

This requires serious consideration. Tagnon, Whitmore, Schulman and Kravitz (1953) related abnormal fibrinolysis in such patients to the metastases and thus to the total amount of carcinomatous tissue in the body, all of which was capable of releasing the lytic agent. Thus a growing carcinoma releasing lysin could be expected to be associated with a progressive shortening of the lysis time. Even so, one would still not expect to find deterioration in patients very recently discharged from hospital and feeling much fitter than before. Moreover, the normal distribution of lysis times from patients where the carcinoma had metastasised has already been discussed in chapter four.

If an increase in the rate of fibrinolysis can be explained by an advance of the underlying carcinoma, the same increase should also be demonstrable in patients examined first as out-patients and subsequently as in-patients: in patients having multiple examinations as in-patients: and in patients having multiple examinations as out-patients.

Table XXVIII below shows the effect of the passage of time on the three patients in the series who were admitted to hospital after an out-patient fibrinolysis assay.

TABLE XXVIII

Lysis times before and after admission to hospital

Name	Fibrinolysis as out-patient P_{100}: P_{50}: P_{10}	Fibrinolysis as in-patient P_{100}: P_{50}: P_{10}	Time interval (months)	Increase (+) or diminution (-) of lysis
S.H.	22: ⁂ : ⁂	48: ⁂ : ⁂	2	-
A.M.	68: 35: 25	77: 80: 69	4	-
D.P.	65: 47: 41	46: 26: 22	5	+

(lysis times in hours)

⁂ observations at different time intervals make comparisons impossible. All lysed quickly.

It would appear that two of the three patients lysed more slowly after admission to hospital and one more rapidly.

The second table in this group, table XXIX, shows the effect of time on the eight patients who had more than one fibrinolysis assay when in hospital. Assays made at intervals of less than two weeks have been excluded, this being the minimum time involved in figure 15. Where more than two in-patient assays were made, the first and the last only have been accepted for inclusion here.

TABLE XXIX

Differences between lysis times of patients remaining in hospital

Name	Fibrinolysis at first hospital examination P_{100}: P_{50}: P_{10}	Fibrinolysis at last hospital examination P_{100}: P_{50}: P_{10}	Time interval (months)	Increase (+) or diminution (-) of lysis
P.A.	96: 59: 48	++: ++: 77	4	-
C.C.	++: 98: ++	++: ++: ++	10	±
W.J.	47: 56: 28	45: 23: 10	5	+
T.L.	70: 35: 22	71: 55: 25	16	-
F.P.	50: 50: 25	50* 20* 7*	1	+
E.A.S.	8: 4: 3	46: 29: 22	¾	-
E.S.	++: 96: 48	6: 6: 4	28	+
W.C.	46: 48: 57	22: 22: 22	1	+

(lysis times in hours)

* the patient died within 48 hours. The figures obtained one week earlier were P_{100} ++: P_{50} 79: P_{10} 55

++ no lysis by 240 hours

It would appear from the table that approximately equal numbers showed an increased lysis time as showed a diminished time. There does not appear to be any uniform shortening of the fibrinolysis time during stay in hospital.

The third table in this group, table XXX, concerns the three patients of the series who had more than one fibrinolysis assay performed as out-patients.

TABLE XXX

Differences between lysis times of patients remaining as out-patients.

Name	Fibrinolysis at first out-patient examination P_{100}: P_{50}: P_{10}	Fibrinolysis at last out-patient examination P_{100}: P_{50}: P_{10}	Time interval (months)	Increase (+) or diminution (-) of lysis
G.T.	72: 55: 144	96: 72: 96	14	?-
W.J.	31: 18: 7	54: 23: 10	3	-
F.V.	17: 17: 17	16: 16: 16	½	±

(lysis times in hours)

There is no evidence to suggest that there is uniform increase of lysis in patients examined at intervals in the out-patient department

Although the numbers of cases are small in these three tables, the evidence is definitely against a uniform group increase of lysis related to the passage of time, such as could be related to increase of the carcinoma. The evidence to be supplied shortly on the relationship of fibrinolysis to the timing of the specimen before the death of the patient also supports the claim that there is no uniform increase of lysis with the passage of time.

THE EFFECT OF SPECIFIC THERAPY

Although it has been claimed that stilboestrol is capable of diminishing fibrinolysis when given to patients suffering from hormone sensitive carcinoma of the prostate (Tagnon, Schulman, Whitmore and Leone, 1953) - and this appears to have been demonstrated here in case 2, which has been described in greater detail - it remains possible that in other patients a reverse effect upon fibrinolysis may result. However, in two patients of the group of seven in figure 15 stilboestrol therapy had already been started before the first assay, and in one no stilboestrol was ever given. In the four others, stilboestrol therapy was begun between the two examinations. Stilboestrol would not, therefore, appear to be responsible for the increase of lysis which was shown in all the patients. Further observations on the effect of oestrogens on fibrinolysis have already been made in chapter four.

FIBRINOLYSIS IN RELATION TO SURVIVAL IN CARCINOMA OF THE PROSTATE

Figure 16 has been prepared so as to examine the relationship of fibrinolysis to approaching death. The first fibrinolysis assay made on each of 30 patients who had been examined within the last two years of life has been plotted in relation to the time in months before death. These points are shown in the figure.

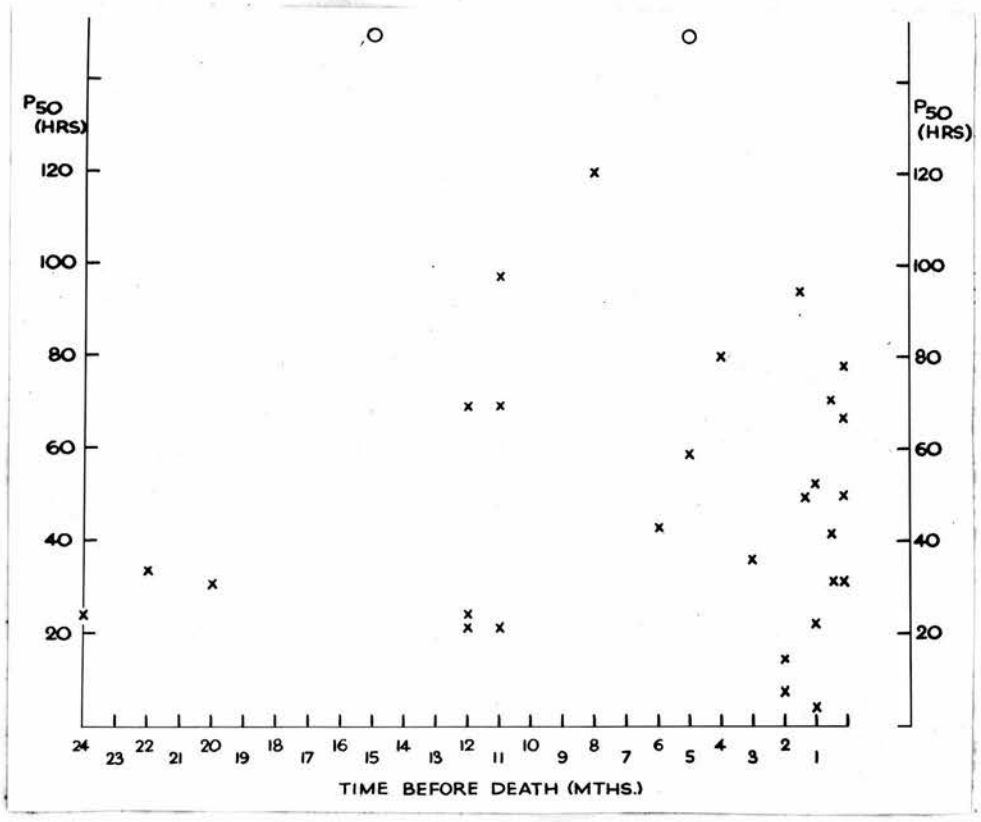


FIG. 16: Scatter diagram showing the relationship of the lysis times of clots prepared from 50 per cent plasma to the time before death. The circles represent two assays where the clots failed to lyse at all.

Analysis of these points has failed to show any significant trend towards increased or diminished lysis as death is approached. Scanning analysis of the scatter diagram is unconvincing and can show different trends according to the inclination of the viewer. More points might have shown a trend one way or the other but there is enough evidence here to show that there is no clear change towards the end of the lives of these patients, despite a high proportion having died of causes attributed to their carcinoma.

DISCUSSION OF THE EVIDENCE OBTAINED AT FOLLOW-UP

The evidence which can be analysed from the follow-up investigations has drawn attention to the frequency with which out-patient fibrinolytic activity is greater than it was previously as an in-patient. This cannot be explained solely on the basis of increasing age, increasing disease or oestrogen therapy. Nor can it easily be related to the imponderables of the last few months of life, even when many of those who died were believed to do so because of the carcinoma.

There is no new discovery about fibrinolysis increasing after exercise, or in relation to emotion, but previous work on these points had not proved that out-patient work must necessarily be avoided. The patients in this series came almost without exception by ambulance, and none of them

walked more than a few steps before the venepuncture. Fearnley, Chakrabarti and Vincent (1960) stated that "diurnal variability, the known effects of exercise, and the possible effects of meals" had made them confine their study to in-patients. With this approach we would now agree.

Within the limitations of the small numbers involved, the evidence is uncertain so far as oestrogens are concerned. Table XVIII shows more patients whose lysis times became faster on oestrogen than became slower. The method as used here is not definitive enough to make a fine assessment of the effect of oestrogens on fibrinolysis and it requires an independent study to assess the part played by oestrogens in physiological states. This is a relevant problem. Current research often associates fibrinolysis with vascular thromboses, and myocardial infarction is much less common in women than in men. Oestrogens would therefore appear to justify study in this context.

CHAPTER SIX

FIBRINOGEN

ANALYSIS OF FIBRINOGEN LEVELS

Fibrinogen estimations were carried out on all specimens of blood removed for fibrinolysis assay. Such estimations were related to the main problem of fibrinolysis but were secondary to it. The plasma fibrinogen levels have been arranged by age and grouped in table XXXI. The figures have been taken from table XXXII and table XXXIII in appendix 2.

TABLE XXXI

Comparison of the mean fibrinogen levels as found in each decade in the main series with the three sections of the control series

Age	No. of cases	Mean plasma fibrinogen	No. of cases	Mean plasma fibrinogen
	Carcinoma of prostate		Benign prostatic hyperplasia	
50-59	7	0.46	2	0.46
60-69	18	0.46	11	0.50
70-79	26	0.54	5	0.44
80-89	6	0.46	2	0.54
	Control malignant		Control non-malignant	
50-59	9	0.52	12	0.37
60-69	16	0.56	18	0.54
70-79	9	0.53	2	0.40
80-89	2	0.72	2	0.27

unit = grams per 100 ml.

If the grand means of the fibrinogen levels in the malignant prostate and benign prostate groups are compared with each other, no difference is apparent, either on visual scanning or after statistical analysis; nor is there any apparent effect of age on plasma fibrinogen level.

It is clear that the group in the control series with malignant disease has a higher fibrinogen average than any other group, but analysis by simple methods was not possible because variance between age groups was significant, indicating that the groups were not homogeneous. However, there is no obvious variation of fibrinogen with age in any group.

Comment:

1. There is no apparent change in fibrinogen level with age.
2. There is a great similarity of mean fibrinogen level in 68 patients with prostatic carcinoma and 20 patients with benign prostatic hypertrophy.
3. Each of the remaining groups lacks homogeneity and cannot be simply analysed. The overall raised plasma fibrinogen levels in the control carcinoma group is in contrast to all other groups, including that of carcinoma of the prostate.

The following two case histories have been included because each patient developed hypofibrinogenaemia.

Case history A

A man J.H. aged 54 was admitted to hospital as a list case on 28.6.56. He gave a history of renal pain over the previous 18 years. In 1938 he had undergone a left nephrolithotomy and a second one had been performed in 1945, this time on the right side. On the present occasion the symptoms were of dysuria and frequency, together with persistent pain in the left loin. His general health was not good. His blood urea was 107 mg. per 100 ml. X-ray showed bilateral staghorn calculi. Surgery was considered his only hope of survival.

Operation: The perinephric space was obliterated and the (left) kidney could only be freed by stripping the capsule. This led to considerable capillary oozing. The largest calculus and the majority of the smaller stones were removed through the pelvis and three were removed through incisions in the renal substance. At the end of the operation the patient's condition was poor, despite blood transfusions.

Two and a half hours after the end of the operation he had made little response to ten bottles of blood. The patient was therefore returned to the operating theatre and the wound partly opened without anaesthetic and packed with two rolls of gauze and three large abdominal packs.

Two hours later (four and a half hours after the operation) the patient was found lying in a large pool of blood. An emergency nephrectomy was then performed in his

bed in the ward, again without anaesthetic. Bleeding was seen to be coming from many small points, including the original incision. Local measures did not stop the bleeding.

At this stage haematological advice was sought, but blood for examination had to be taken from the femoral artery. The specimen so obtained was divided into parts for subsequent examination.

The whole blood formed a gelatinous clot which retracted poorly. The Wolf (1954) method (see chapter two) for fibrinogen assay suggested that fibrinogen depletion was half that of the control, although it is an unconvincing test at this level of difference. The Quick one-stage prothrombin time was 26 seconds (control 13 seconds).

On this evidence 4.5 grams of the fibrinogen fraction of human plasma was reconstituted with water and given intravenously. Calcium gluconate was injected and more fresh blood given. A total of 42 pints of blood was given during the operative and immediate post-operative period. Some improvement took place and significant bleeding did stop, but too many aids were given for any one of them to be recognised as the most important. The patient lived a further five days, dying then of the circulatory and general failure from which he had never wholly recovered. The histology of the kidney which was removed showed relatively little recognisable renal tissue, and autopsy showed the remaining staghorn calculus in the other kidney.

The following observations were made on the blood sample removed at the time of the emergency.

Fibrinolysis: The whole blood clot showed no sign of lysis when observed at 24 hours after incubation at 37°C. Plasma assay, using oxalated plasma, clotted by thrombin and then by the standard method of this work, showed no lysis in any tube by 24 hours.

Fibrinogen estimation: (using Podmore's method, 1959)

Pre-fibrinogen therapy sample, 0.13 g. per 100 ml. Post-fibrinogen therapy sample 0.23 g. per 100 ml.

Correction experiments on the prolonged Quick one-stage prothrombin time. The patient's time of 26 seconds was reduced to 20 seconds by "factor V" plasma, to 19 seconds by serum, and to 21 seconds by added bovine fibrinogen. The methods used were in accordance with Biggs and Macfarlane (1953a). The platelet count was 85,000 per c.mm. (lower range of normal = 150,000/c.mm.).

Interpretation: It is almost impossible to interpret these findings with accuracy because the primary cause of the bleeding was not established. If it was due to local causes arising from difficult surgery, then the subsequent haematological findings may have been caused by excessive use of blood transfusions. This has been described before as leading to multiple deficiencies

of coagulation factors but only rarely to hypofibrinogenaemia (Krevans and Jackson, 1955). On the other hand, it remains possible that this was an example of defibrination due to intravascular clotting. It is of interest to note that the blood removed from the patient when he was severely shocked showed no evidence of fibrinolysis.

Case history B

This is one case of obstetrical bleeding and is only relevant because of the associated hypofibrinogenaemia. The case will be described briefly and has been included only for the sake of completeness.

A woman N.W. aged 22 developed an ante-partum haemorrhage at the 35th week. There was moderate bleeding and bruising at venepuncture sites. She vomited half a pint of blood and vaginal bleeding increased. Plasma fibrinogen was 0.11 g. per 100 ml.

She was treated with two bottles of triple strength plasma without it making any apparent difference, but this therapy was concurrent with the delivery. After the placenta was delivered fibrinogen fraction from human plasma was injected and this appeared to be responsible for stopping all abnormal bleeding. The child died shortly before delivery.

Comment: The triple strength plasma may have been ineffective because the placenta was still in the uterus, and the fibrinogen may have gained the credit for the recovery because the placenta had by then been delivered and there was no further

source for thromboplastin release into the maternal circulation. The source of the hypofibrinogenaemia in such patients is widely believed to be due to intravascular coagulation secondary to release of thromboplastic substances into the blood stream from the placenta (Page, Fulton and Glendening, 1951).

GENERAL DISCUSSION

The special association of serious bleeding with prostatic carcinoma has been described from time to time (Jürgens and Trautwein, 1930: Marder, Weiner, Shulman and Shapiro, 1949: Seale, Jampolis and Bargen, 1951: Cosgriff and Leifer, 1952) but Tagnon, Whitmore and Shulman (1952) appear to have been the first to give prominence to fibrinolysis as the cause of the bleeding. They first defined fibrinolysis as the dissolution of a whole blood clot within 24 hours when kept at 37°C and then described two cases of spontaneous bleeding associated with fibrinolysis, but the plasma fibrinogen level was not known in either. In an addendum they reported the case of a third patient, showing fibrinolysis where there was also fibrinogen depletion. When they deliberately looked for fibrinolysis in 14 other patients with carcinoma of the prostate, they failed to find it. These patients, who were between 53 and 78 years old, were not bleeding at the time of the examination.

In the subsequent year Tagnon, Whitmore, Schulman and Krawitz (1953) gathered together all their cases, including two new ones which they described in detail, and published a report of fibrinolysis in six out of 48 patients with carcinoma of the prostate. This may be the largest published series. Five out of these six patients had bleeding manifestations believed due to the fibrinolytic enzyme and in at least three of them there was also a deficiency of plasma fibrinogen as

well as a prolongation of the prothrombin time. The carcinoma had metastasised in all the patients who bled. In vitro experiments showed that the carcinomatous tissue both from the primary site and from metastases had considerable fibrinolytic activity and digested fibrinogen, prothrombin and factor V in addition. The writers suggested that such tissue juice escaping into the blood stream was the immediate cause of the fibrinolysis, the fibrinogen depletion and the prolonged prothrombin time which they found in vivo. This implied a pathological type of fibrinolysis.

Much of the work which has been reported in the preceding pages here has resulted from the stimulus of Tagnon's observations. The pattern of the work first began to take shape from a desire to clarify our understanding of what happened to one particular patient in whose prostate a carcinoma had developed. He almost died as a result of hypofibrinogenaemia in the course of prostatic biopsy and orchidectomy, yet neither at that time nor for some years subsequently did the patient show any unusual activity of plasma fibrinolysis. When it comes to explaining the crisis which occurred, one can find no equivocation in the claim by Tagnon and his colleagues that fibrinolysis is the essential component in such patients, and that it is fibrinolysis which is associated with intravascular digestion of fibrinogen and other coagulation factors. This seems to prevent Tagnon's theory being a suitable explanation of the acute emergency in our patient where there was no evidence of abnormal fibrinolysis at the critical time.

It would be unexpected to find the lives of such patients threatened by serious bleeding from two different mechanisms, one resulting from fibrinolysis and the other not even associated with fibrinolysis, yet both associated with hypofibrinogenaemia. Therefore it seems profitable to discuss whether the relationship of fibrinolysis to fibrinogen depletion has been correctly interpreted up to the present time.

There are several problems to be solved. Is fibrinogen depletion necessarily related to fibrinolysis at all? Is either fibrinogen depletion or fibrinolysis dangerous when occurring without the other, or must they occur together to cause clinical bleeding? Is abnormal fibrinolysis an extreme form of the physiological or is it ever primarily pathological? These points will be discussed.

Laboratory work has demonstrated time and time again that plasmin digests fibrinogen as well as fibrin. This observation is not questioned, but great uncertainty is felt about its application in vivo. Experimental studies very commonly use streptokinase or chloroform as a means of plasmin production, but neither of these two substances is physiological and there is no validity for assuming without proof that they re-create physiological states, or even pathological conditions of a non-specific type. A certain amount of support for their possible validity comes from plasma fractionation procedures but these also are subject to artefact conditions.

Doubt cast upon the *in vivo* application of so much good experimental work is more than an academic exercise because experimental work on plasmin is the only justification for attributing clinical hypofibrinogenaemia to fibrinolysis except in cases where it is claimed there is a pathological lytic agent which acts directly. The management of the patient depends upon one's interpretation of the mechanism underlying clinical bleeding. If the low plasma fibrinogen in a patient is due to digestion of fibrinogen by plasmin in the circulation, then the sensible treatment is to inhibit or remove the digesting agent. This can be done, apparently with success, by the use of the soya bean trypsin inhibitor, E-aminocaproic acid and other preparations. If the low fibrinogen level is not due to digestion but is due to excessive consumption of plasma fibrinogen by premature clotting, which is the most important alternative explanation, then anticoagulants such as heparin would appear to be the rational therapy, for maintenance and prophylaxis, if not for the acute episodes. Successful treatment with heparin has been reported in purpura fulminans associated with hypofibrinogenaemia (Little, 1959).

The evidence against *in vivo* digestion of fibrinogen is fairly strong. It has often been observed in clinical-laboratory work that the plasma fibrinogen level has been normal in the presence of very active fibrinolysis. This has been shown after exercise, in cirrhosis of the liver, after

obstetrical accidents and in cadaver blood (if this may be mentioned here) and these have been documented in the section devoted to plasmin in chapter one. Fearnley (1956) stated that prolonged incubation of citrated plasma, whether diluted or undiluted, from patients with cirrhotic livers gave no reduction of fibrinogen content even when fibrinolytic activity was great.

The work here amply confirms this apparent dissociation of fibrinogen level and fibrinolysis. The nine fastest rates of lysis in the whole series of 206 patients were associated with fibrinogen levels of the order of 0.26 to 1.06 g. per 100 ml. (table X). Even the patient (case 2) whose fibrinolysis was very active at room temperature, giving some justification for suspecting a pathological element to the fibrinolysis, had raised plasma fibrinogen levels of 0.49 and 0.62 g. per 100 ml. on two occasions when lysis was very active. Moreover, the lytic potential in the same patient was able to survive in the plasma in vitro over the course of days without any apparent detriment to the fibrinogen, but as soon as the same fibrinogen was converted to fibrin, lysis took place rapidly.

Thus the evidence in favour of naturally occurring fibrinolysis being specific for fibrin seems better than the evidence in favour of its also being fibrinogenolytic. There are, however, at least two alternative explanations for the same phenomenon. The first is that the fibrinolysin is

held inactive in a reversible combination by plasma antilysins (Hussey and Northrop, 1923) but is released from the antilysins by its adsorption on to fibrin when clotting occurs. There is much to commend this explanation. The increase of lytic activity found on some occasions in the plasma of patients might then be explained by a return of some of the lysin to the plasma on its release from the fibrin to which it had been adsorbed and which it had only recently digested. Antilysins would hold the released fibrinolysin inactive in the plasma until more fibrin was presented to it or until the titre fell again in the course of body metabolism.

A second explanation, which is entirely theoretical, is that the lytic activity in the plasma may remain at a precursor stage (plasminogen) until it is activated directly, or more likely indirectly, by some component of the clotting mechanism, both <u>in vivo</u> and <u>in vitro</u>. Such activation would clearly occur too late for the demonstration of fibrinogenolysis, even if the fibrinolysin (plasmin) were also fibrinogenolytic, because the fibrinogen would already have been converted to fibrin. There is nothing mutually incompatible about these two explanations because neither is complete in itself. They could occur together. The work of Fearnley and Ferguson (1958) would favour the clotting process having an inhibitory effect on fibrinolysis and is evidence against the second of the explanations. Further elaboration of theory is not justified without

experimental work at the fundamental level of what coagulation factor or para-factor of coagulation could influence fibrinolytic events. Such work cannot be performed easily without refinement of the individual components of the fibrinolytic system greater than is at present possible.

It is important to know whether fibrinogen depletion or fibrinolysis is dangerous to the patient when present as a single finding. Congenital deficiency of fibrinogen has been described by Henderson, Donaldson and Scarborough (1945) and by Alexander, Goldstein, Rich, Le Bolloc'h, Diamond and Borges (1954) who investigated very carefully some of the basic aspects of coagulation in their patients. They described two boys and one girl who suffered from repeated spontaneous and post-traumatic haemorrhages into skin, muscles, joints and loose tissues resulting from severe fibrinogen depletion as an isolated abnormality. Clinically the condition resembled haemophilia.

In the partial deficiencies of fibrinogen, the actual plasma level at which bleeding can occur may not be of primary importance. A slight injury with absent fibrinogen may be as serious as a more severe injury with a higher but still deficient fibrinogen. Patient E.S. (case 1) had stopped

bleeding at a plasma fibrinogen level of only 0.08 g. per 100 ml. There was no demonstrable fibrinolysis at the time. It is in the determination of the critical level of plasma fibrinogen below which bleeding will occur in the appropriate circumstance that secondary fibrinolytic activity could perhaps play an important clinical part, and, being present, could perpetuate bleeding. However, it seems correct to believe that fibrinogen deficiency of sufficient degree can be responsible for serious bleeding even in the absence of other abnormalities.

The reverse situation of unusually active fibrinolytic activity without fibrinogen depletion is probably seen relatively commonly by anyone performing frequent fibrinolytic assays, but the part played by active fibrinolysis in causing or perpetuating bleeding is not really established. It has been claimed from time to time (Tagnon, Whitmore and Shulman, 1952) that lysis of a whole blood clot by 24 hours at 37°C may be taken as evidence of (abnormal) fibrinolysis. In such cases the whole plasma clot, which is much more easily observed for complete lysis, usually lyses a little before the whole blood clot. In the present main series of 68 patients, six whole-plasma clots lysed by 24 hours (three of these being from out-patients), yet in only one of these was there any bleeding tendency, and it was not alarming. One may also cite Macfarlane in 1937 and our own work reported here

as examples of fierce fibrinolysis in patients during and after surgery when the surgeon had found haemostasis to be normal. It is clear that active fibrinolysis by itself need not be associated with bleeding.

If one proposes that bleeding has resulted from a combination of hypofibrinogenaemia and fibrinolysis there seems to be no easy way of assessing individual responsibility. Some success has been claimed from the use of anti-fibrinolytic therapies, but there has usually been an absence of the dramatic success which has so often attended the administration of intravenous fibrinogen in cases of acute hypofibrinogenaemia. Nilsson, Sjoerdsma and Waldenström (1960) described an ill man who had acute promyeloblastic leukaemia and was bleeding. His plasma fibrinogen level was 0.07 g. per 100 ml. and his whole blood clot lysis time was 15 minutes. He was treated with E-aminocaproic acid and the haematuria and melaena ceased simultaneously, although he died a few days later.

The combination of fibrinolysis and fibrinogen depletion has often been described in association with carcinoma of the prostate. Tagnon, Schulman, Whitmore and Leone (1953) described a close follow-up lasting 150 days of a 58 year old negro in whom the carcinoma had metastasised. They noted that fibrinolysis was intermittent but when present it was associated with a simultaneous fall of plasma fibrinogen and an increase of the prothrombin time. In

addition there were fluctuations in the platelet count which were roughly parallel with the fluctuations of the fibrinogen level. Oestrogens appeared to cause a beneficial effect on fibrinolysis, on the fibrinogen level and on the prothrombin time. Testosterone was detrimental. Two cases of carcinoma of the prostate were described by Crane, Ware and Hamilton (1955). In the first, afibrinogenaemia developed seven hours after trans-urethral resection of the prostate, but the only evidence for fibrinolysis in this case was obtained preoperatively. Operative haemostasis was normal. Their second case showed both hypofibrinogenaemia and fibrinolysis. Kellock and Gallagher (1958) described a case of metastatic carcinoma of the prostate where both fibrinolysis and a fibrinogen level of 0.10 g. per 100 ml. (and a platelet count of 40,000 per c.mm.) were present at the same time. Lombardo (1959) described an example following trans-urethral resection of a malignant prostate. Four hours after the resection the plasma fibrinogen level was 0.04 g. per 100 ml. with complete lysis of the patient's (hypofibrinogenaemic) clot in one hour. Stefan, Chrobák and Groh (1960) reported the case of a man with metastatic carcinoma of the prostate who presented with unilateral bleeding from a kidney and was found to have hypofibrinogenaemia (0.14 g. per 100 ml.) and fibrinolysis.

The work of Tagnon and his colleagues has so impressed itself on those interested in fibrinolysis that it is difficult to determine whether the frequent publication of

cases of fibrinolysis in carcinoma of the prostate represents a false incidence due to the publicity given to the problem, or whether in fact there is really an incidence in carcinoma of the prostate which is greater than in other pathological states. It is also possible that bleeding which has resulted from fibrinogen depletion has led to many of the studies which have shown fibrinolysis.

The following are some published examples of fibrinogen depletion from causes other than carcinoma of the prostate. Many of them have been associated with fibrinolysis. Coon and Hodgson (1952) described a patient where the fast lysis and fibrinogen depletion followed open cardiac massage, and it seems possible that tissue juices were released into the blood stream in the process. They also described a patient who had an operable carcinoma of the bronchus. His plasma showed fibrinolytic activity "in inverse relationship" to changes in plasma fibrinogen. When the tumour was removed surgically, fibrinolysis and plasma fibrinogen both returned to the levels found on admission. Depression of fibrinogen has also been found after surgery for carcinoma of the kidney, where there was no obvious fibrinolysis (Phillips, Skrodelis and Furey, 1959); after benign prostatectomy, associated with fibrinolysis (Scott, Matthews, Butterworth and Frommeyer, 1954: Lombardo, 1959); after lobectomy (Chalnot, Michon and Mme. Lochard, 1952: Baumann, 1952); after pulmonary resection, associated with fibrinolysis (Soulier, Mathay, Le Bolloc'h, Daumet and Fayet, 1952); after abortion by intra-uterine

injection of hypochlorite solution, associated with fibrinolysis (Soulier, Petit and Le Bolloc'h, 1952); associated with carcinosis of the bone marrow having metastasised from carcinoma of the gall bladder in one case and from the stomach in another, fibrinolysis being temporarily observed (Braun and Horányi, 1951); associated with pre-terminal carcinoma of the stomach and an undiagnosed case of hepato-splenomegaly (Bennike and Müllertz, 1952); in acute myelogenous leukaemia together with increased fibrinolysis (Cooperberg and Neiman, 1955). Phillips, Rowley and Habif (1956) demonstrated some hypofibrinogenaemia often with fast lysis in nine cases during surgery, usually of a major type, including aneurysm resection, radical mastectomy, bile duct surgery and partial gastrectomy in a patient suffering from cirrhosis of the liver. Many examples have been published of fibrinogen depletion associated with premature separation of the placenta (Page, Fulton and Glendening, 1951) where the presentation is usually acute, and with retention of a dead foetus (Pritchard and Ratnoff, 1955) where the development of fibrinogen depletion is usually slow. Hypofibrinogenaemia associated with amyloidosis (Bowman, 1958) is presumably due to failure of liver production. Haemolytic transfusion reactions have led to hypofibrinogenaemia, without fibrinolysis (Jackson, 1956) and massive compatible blood transfusions may rarely be followed by hypofibrinogenaemia (Krevans and Jackson, 1955). To this list we would add our case of J.H. (case A, chapter six) where hypofibrinogenaemia followed major operative and transfusion interference because of bilateral staghorn calculi in the kidneys.

The terminal few months of life of the patient E.S. (case 1, chapter four) can perhaps be compared to those of the negro followed so closely for 150 days by Tagnon and his colleagues. During this period, which was without operative interference, the patient E.S. maintained active lysis and a moderate fibrinogen depletion, in contrast to his initial presentation during operative surgery. At that time there had been a dramatic hypofibrinogenaemia, but after treatment it was followed by haematological good health for some years until the malignancy escaped from control. It is submitted that the evidence in this patient towards the end of his life is consistent with a hypothesis of chronic spontaneous secretion of clotting substances into the blood stream, with continuous consumption of fibrinogen at such a rate as to overtax his liver in its capacity as fibrinogen producer. Such a chronic hypercoagulation is not inconsistent with the leg thrombosis from which the patient suffered and with the pulmonary embolus from which it seems likely that he died. It is also reasonable to assume that most of the products of intravascular coagulation would be removed by the active fibrinolysis which was so easily demonstrated.

Evidence in favour of intravascular coagulation is by its nature difficult to prove. The only indicator of coagulation is the clot itself, and intravascular fibrin is virtually always removed by lysis before it can be demonstrated, except in rare and often catastrophic examples (e.g. McKay,

Mansell and Hertig, 1953). All that may be left with which to demonstrate intravascular coagulation is a demonstration of changes such as are consistent with coagulation. Such a concept of the occurrence of intravascular coagulation is in keeping with the interpretation of the findings made by Rapaport and Chapman (1959) in a patient suffering from carcinoma of the prostate. This patient showed hypofibrinogenaemia, diminished factor V and true prothrombin, and a moderate thrombocytopenia (but no definite increase in fibrinolysis).

If one supposes that intravascular coagulation has been occurring due to a spill-over of thromboplastic prostatic tissue juices, the fibrinogen depletion is explained but not the fibrinolysis. Because the fibrinolysis which was demonstrated in E.S. (and in other patients in the main series and in the controls) seemed to have no obvious peculiar characteristics of behaviour it is suggested that the fibrinolysin was of a physiological type. A hypothesis has already been put forward that adsorbed fibrinolysins may become available for re-use after lysis has taken place, and could thus be responsible for a rise in plasma titre in any case where intravascular coagulation has occurred in the presence of a normal fibrinolytic system. If, however, the fibrinolysis which was demonstrated in the laboratory had characteristics suggestive of "pathological" fibrinolysis (for example strong activity maintained at room temperature) this might be attributable to tissue juice being present in excess

in the blood stream and acting directly on the fibrinolytic mechanism. Such an occurrence in an obvious form, presuming it occurs at all, seems to be very rare. The only patient in the present series where "pathological" fibrinolysis may be suspected had a plasma fibrinogen level slightly above normal (case 2).

It is interesting to speculate whether the heat stability of the fibrinolytic system in case 2 could have been due to the presence of the tissue activator of Astrup and Permin (1947) arising perhaps from the prostate. Ordinary plasma activator is heat labile but tissue activator is heat stable and is known to exist in the prostate (Rasmussen and Albrechtsen, 1960) as well as in most other tissues. It is felt that an investigation into the heat stability of the activator in the plasma of patients suffering from carcinoma of the prostate might be a profitable line of research, designed to prove the existence of a direct acting pathological fibrinolysin not ordinarily demonstrable in the plasma. Without a study of the thermal range of physiological fibrinolysis, particularly when very active, it would be unwise to assume that heat stability is of itself an indication of a pathological type of fibrinolysis.

There still remains an unanswered problem of how hypofibrinogenaemia can sometimes be found with fibrinolysis and other times without it. In addition to this question involving the relationship between clotting and fibrinolysis, a point which has been discussed already, there are also matters

of fact which may have been misinterpreted from time to time. It is well recognised by those who have had to deal with the serious emergency of acute hypofibrinogenaemia that there is little time for studied research during the emergency and the first specimen of blood may be left in the laboratory before the assay for fibrinolysis is set up, for long enough to destroy some of the physiological lysins, particularly if the specimen is not kept refrigerated (Fearnley, Revill and Tweed, 1952). During the period of hypofibrinogenaemia the use of homologous fibrin, or of an alternative substrate, is obligatory. Specimens removed some hours later after successful therapy with intravenous fibrinogen may still show a low fibrinogen content but if the patient is recovering there is no reason why fibrinolysis should still be active. It remains possible that increased physiological fibrinolysis was missed for these reasons in the case of E.S. when first seen, and in some other published cases.

A decision whether abnormal fibrinolysis is an extreme form of the physiological or is primarily pathological must remain at the moment one of opinion. To a large extent it is the problem of whether fibrinolysis can be divided into primary and secondary types. It is hard to imagine that the short, sharp episodes of fibrinolysis associated with routine surgery are anything but secondary, and it is of interest to note how

active lysis can be on such occasions. Similarly adrenalin has induced very active lysis in some subjects, although the example quoted here using a smaller dose was less dramatic. While most of the examples quoted throughout this work are suggestive of a physiological or secondary fibrinolysis, the one exception to this (case 2) was so obviously different as to draw attention to itself by lysing the clot completely at room temperature. This observation was controlled because almost all the other samples were treated similarly in the course of fibrinogen assay, and in none other did this obviously occur. It is suggested that this represents the one and only fair example of what may be called pathological fibrinolysis out of assays on 206 patients, 68 of these being diagnosed as having a carcinoma of the prostate.

If one may summarise the hypothesis here, it is that fibrinolysis can be primary or secondary. The secondary type is very common and is related in some unproved way to blood coagulation and to adrenalin. It can be called physiological fibrinolysis. The primary type of fibrinolysis is much less common and results from the escape of fibrinolytic juice into the circulation in sufficient quantity to be measurable by its direct action on the fibrinolytic mechanism.

The tissue juice of carcinoma of the prostate, being both thromboplastic and fibrinolytic, could be responsible for several syndromes, all of them rare. The first would be that usually provoked by local surgery of sudden flooding of the

circulation with prostatic thromboplastin, leading to acute hypofibrinogenaemia and to primary or secondary fibrinolysis. All this is of short duration and is followed by death of the patient or relatively complete recovery. It would be easy on such occasions to miss any associated fibrinolysis both because of the short duration of the episode and because of technical difficulties when dealing with acute hypofibrinogenaemia.

The second syndrome would occur where thromboplastic tissue juices reach the blood stream as a small chronic spill-over, usually unprovoked by interference. This would lead to a lowered fibrinogen level with secondary fibrinolysis which presumably would mop up the end product of intravascular clotting or would itself be the result of the mopping up. The amount of tissue juice would be too small to be effective by a direct action on the fibrinolytic mechanism.

The third syndrome would result from the escape of tissue juice which by its quantity or its quality would act directly on the fibrinolytic mechanism and could be demonstrated to be different from the physiological fibrinolysis by, for example, its physical properties. This need not be associated with fibrinogen depletion. It is possible that the second and the third syndromes would represent equilibria between the noxious agent and the body defences. The capacity of the liver to regenerate fibrinogen is particularly important in this context and observations on the biological half life of such fibrinogen would be interesting (see Hammond and Verel, 1959).

Perhaps these suggestions are too complicated. It may be that the same prostatic juice is both thromboplastic and fibrinolytic at one and the same time in vivo, for this would not even demand a relationship between coagulation and lysis. This seems to cut across the concept of physiological interrelationships.

By such hypotheses all the peculiarities of the patients described relating to fibrinogen depletion and fibrinolysis are explained away. It would appear to be necessary to examine more patients similarly affected, both in an emergency and in the chronic phase, in order to test the hypothesis. The explanation which has been given is applicable in its entirety only to tissue which is simultaneously thromboplastic and fibrinolytic. We have demonstrated this to be the case in carcinoma of the prostate, but it would be interesting to know if the combined thromboplastin and fibrinolytic activity is equally apposite to other lesions developing elsewhere in such places as have only rarely been associated so far with fibrinolysis and hypofibrinogenaemia. There is much in published work to suggest that this would be so.

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Results have been presented showing an increase of lytic activity with age, and there is a close parallel between this and the increase of latent prostatic carcinoma with age

as published by Franks (1954). He found a progressively increasing incidence of histological carcinoma in his series of autopsies on men from the age of 40 until the ninth decade, when he found latent carcinoma in almost all the prostates examined. Frank's work created the problem which made a choice of controls for carcinoma of the prostate so difficult, even although one did not expect a carcinoma which was only latent to influence the results. It was the reason why the controls were divided into those where there was known benign prostatic hypertrophy, those where there was known malignancy elsewhere than in the prostate, and those admitted to hospital for reasons other than malignancy. It was decided not to use women as controls for a prostate series because it seemed desirable to narrow down the controls as far as possible. Variable factors due to sex differences would add an unknown which might prove difficult to analyse.

The three control groups and the carcinoma of prostate series were remarkably alike in their display of increasing lysis with age, and when all are taken together the results hold even greater authority. Clearly the increased lysis is not a feature of clinical carcinoma of prostate, nor of recognised malignancy elsewhere in the body, but is a phenomenon common to all men in hospital taken as a group. In this respect the control series has not acted as a control. To test the observation further it would now be profitable to examine a female series, the significant observation in this

context being the presence or absence of a slope of increasing lysis with age. It should be relatively easily assessed.

The range of lytic activity shown by the various age groups never approached the levels associated by many with clinical bleeding. From the age of 40 to 80 the mean lysis time of the 50 per cent clot shortened progressively until it was approximately halved (figure 7). Even at the age of 80 the mean time in hours was greatly in excess of anything associated with abnormally fast fibrinolysis. These observations appear to contribute a point of fact to the study of the process of ageing in men, to which fibrinolysis has so far contributed very little.

No large published series of patients examined for fibrinolysis and covering a wide range of ages has been discovered. Albrechtsen (1957) stated that no correlation could be established between the stable activator concentration and age or sex. Fearnley and Lackner (1955) found no difference in fibrinolysis related to age between 20 and 40 years. Sawyer, Fletcher, Alkjaersig and Sherry (1960) stated that they found no correlation of fibrinolysis with age in the population they studied but did not give details. Buckell and Elliott (1959a) found a higher level of fibrinolytic activity in the younger age group of a series of men, all of whom were under 50 years old.

Some other changes with age have been observed in the blood, one of them being the increasing concentration of antihaemophilic factor reported by Cooperberg and Teitelbaum (1960). These authors felt that their finding was a manifestation of hypercoagulability predisposing to thrombosis. Such a claim appears at first sight to be in contradiction to the finding of increasing fibrinolysis but the complexity of the problem of hypercoagulability and fibrinolysis is apparent and it is not considered that the opinion of Cooperberg and Teitelbaum is necessarily in conflict with our own. It does seem uncertain, however, whether an increased concentration of antihaemophilic factor can be regarded as ipso facto evidence of hypercoagulability.

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It seems appropriate to end this discussion by a critical appraisal of the results which have been presented, lest it could be claimed that they are a false product of the method used to create them. Other results might have been possible using different methods.

The dilution method for demonstrating plasma fibrinolysis has had its critics. Von Kaulla and Schultz (1958) declared that the dilution method was not always correct. They found that diluted plasma was less sensitive than undiluted plasma to the fibrinolysis induced by pyrogens. Sherry, Lindemeyer, Fletcher and Alkjaersig (1959) found that

the Fearnley clot lysis technique was cumbersome and less sensitive than the plasma euglobulin method and found the several dilutions of plasma required to be a further disadvantage.

The method used here has also been found to be cumbersome and time consuming, but this does not condemn it. A serious criticism which can be levelled against the method is the unpredictable times at which the clots lyse. Nothing short of an hourly or two-hourly day and night vigil for a matter of months on end would have allowed the assays reported here to be observed in the optimum way and it is not surprising that Lackner and Goosen (1959) devised a camera to photograph the clots in a modified version of the test. The extremes of shape of the curves shown in figures 3, 4 and 5 show the need for caution if single plasma dilutions are to be used for presenting results, but as the test was carried out here the lysis time of the 50 per cent clot seems to be superior to all others and the clear result obtained in this work from the analysis of a large series does itself defend the use of the method.

It is interesting to note that Fearnley and Tweed (1953) found that lysis was sometimes apparent in thrombin-clotted plasma in a 1:4 dilution when it was not apparent in greater dilutions. They attributed this to an inhibiting effect of thrombin and proceeded to demonstrate this by an appropriate experiment. Later in the same table the reverse situation occurred using plasma from other subjects, and only

the dilute clots lysed. Fearnley and Tweed attributed this to the diluting out of a serum plasmin inhibitor. They may well have been correct, but two such variations of pattern apparent in the same table, taken together with our own findings, do show that there are difficulties associated with the test which makes it difficult to interpret and present. It is not possible to entertain the belief that the test is analytic of any one fibrinolytic component.

Here perhaps lies the strength of the method. It represents, within limits, the final common pathway of all influences concerned with fibrinolysis. Only the rising pH is obviously unphysiological, but this does not seem to affect actively lysing plasma to any obvious extent. If it is wished to demonstrate an effective fibrinolytic influence in a group of people, the principle of the dilution method appears ideal, although in practice it may have disadvantages. It would seem that the observation of increasing lysis in men with the passage of years is complete in itself and needs no qualification.

Without special study it is impossible to venture on the problem of which fibrinolytic component makes the lysis time shorter with increasing age. It could be an increase in activator or a diminution of plasmin inhibitor. No comment is made about the possible function of the increased fibrinolysis. Its relevance to the problem of vascular thrombosis is obvious.

The method used lacks the refinement and accuracy required to show slight variations of lytic activity except in most rigidly controlled trials. Where the variation expected is slight it would seem preferable to use the same subjects repetitively as Fearnley himself has done from time to time. On the other hand when one is using only one assay from each of many patients a more refined method might create its own problems in interpretation due to the very wide variation which appears to exist in physiological fibrinolysis and the superiority of such a method might thereby be diminished.

Whereas the clear finding of an age difference was obtained from analysis of lysis times which were within the normal range, no clear finding arose from study of the abnormally short lysis times. Although a relatively large series of carcinoma of the prostate has been presented here, support for the importance of fibrinolysis in this condition has not been obtained. The problem at this stage is really whether an alternative method of assay might have given a significant incidence of abnormal results. This is not felt to be likely. Observation of the exact time when lysis of clots occurred after a short incubation was very much more accurate than when lysis was delayed. It was only in the latter case that night hours passed without any observations being made. It is felt to be probable that other methods of

fibrinolysis assay, such as the euglobulin lysis time or liberation of radioactive iodine from clots, would have shown the greatest overall activity in exactly the same patients as here.

SUMMARY

A review has been presented of the extensive literature concerning the aseptic dissolution of blood clot, or fibrinolysis. Study of such work draws attention to a widespread uncertainty about the physiological role of fibrinolysis. It can be seen also how frequently the assumption has been made, without proof, that fibrinolytic activity has been the primary cause of bleeding episodes when the evidence goes no further than to demonstrate active fibrinolysis during bleeding.

Differing patterns of results have been obtained from assays of fibrinolysis in 206 patients, using a technique requiring serial dilutions of chilled plasma with subsequent incubation. Analysis has shown that the most comparable figures are obtained from the lysis times of clots prepared from 50 per cent plasma in buffer. With a view to assisting the interpretation of results a study has been made of the effect on the test of environmental factors and drugs.

68 cases of carcinoma of the prostate comprise the main series in this work, the diagnosis being confirmed histologically in 44 of these. The more important case histories have been given in detail.

A control series of 138 male patients in hospital has been divided into three sub-groups for analysis, these being of benign prostatic hyperplasia, miscellaneous malignant

conditions and miscellaneous non-malignant conditions. All groups, including the main series, taken separately and together, show the same statistically significant increase of fibrinolysis with age. The lysis time of the 50 per cent clot is approximately halved between the ages of 40 and 80.

There is no statistically significant difference in fibrinolytic activity between the main series of carcinoma of prostate and the control group. Nor has any certain effect on fibrinolysis been observed associated with metastatic spread, oestrogen therapy or bilateral orchidectomy in appropriate cases of carcinoma of prostate in the main series.

It has been shown here that rapid fibrinolysis in vivo need not be accompanied by fibrinogen depletion or bleeding. In addition, a case of serious bleeding in carcinoma of the prostate reported here in detail has shown fibrinogen depletion without demonstrable fibrinolysis at the time of the crisis.

These observations have been used to support the hypothesis that the abnormal bleeding which sometimes occurs in carcinoma of the prostate is not primarily due to fibrinolysis but is due to a consumption of coagulation factors, including fibrinogen, in the course of pathological intravascular

clotting. The clotting factor may arise from prostatic tissue, laboratory work having shown the presence of thromboplastic as well as a more generally recognised fibrinolytic activity in both benign and malignant prostatic tissue.

When rapid fibrinolysis has been found in this series it has almost always been attributed to the variations of a physiological system, but no claim is made that pathological fibrinolysis cannot occur in association with carcinoma of the prostate. In one case the fibrinolysin was sufficiently heat stable as to suggest that it might have been a pathological fibrinolysin.

APPENDIX ONE

The case of E.S. (case 1)

On February 13th, 1956, a man aged 71 presented with haematuria which had developed over the previous week. He gave a history of unremitting backache over the previous six months and of scalding and frequency of micturition. The liver was palpable 1½ inches (3.8 cm.) below the costal margin in the mid-clavicular line. He had a large fixed prostate gland which was thought to be probably malignant. By the time of his admission to hospital on March 6th the haematuria had stopped spontaneously.

Investigations

A few of the haematological investigations have already been presented in table XI. Radiographs of the pelvis did not show any evidence of bony metastases, but an intravenous pyelogram revealed a moderately severe left hydronephrosis and a lobulated filling defect on the bladder base.

As an initial therapeutic procedure bilateral orchidectomy was performed, and a biopsy of the prostate was also taken, using the perineal approach. This ultimately confirmed the presence of an anaplastic carcinoma of the prostate. After the biopsy had been removed all the traumatized tissue began to bleed freely, and the patient developed peripheral circulatory failure. Post-operatively the perineal wound continued to bleed, and his condition deteriorated. No improvement was observed as a result of

the emergency transfusion of three bottles of stored blood over four hours: indeed, he began to develop spontaneous subcutaneous bruising around the shoulder-girdle and arms. At this stage it was reported that the original specimen of venous blood removed for emergency cross-matching had still failed to clot. The syndrome was fully investigated by the following laboratory tests.

1. Failure of blood to clot. The specimen of blood removed immediately after the operation had not been in contact with an anticoagulant, and yet it appeared to remain completely fluid apart from a very minute clot which comprised little more than a few fibrin strands lying loose within the specimen. This tiny clot did not increase in size thereafter. Indeed, it became less visible during the succeeding five hours. This apparent failure of clot formation as observed in the blood transfusion laboratory suggested the diagnosis of fibrinogen depletion. The following tests confirmed the presumptive diagnosis.

2. Thrombin and whole blood. Since thrombin is a specific indicator of the presence of fibrinogen, given the absence of inhibitors, 0.2 ml. of commercial thrombin (Maw, 50 N.I.H. units per ml. of normal saline) was added to the same volume of oxalated whole blood. When assessed some 60 seconds later a very poor clot had formed. It was small in size, occupied a mere fraction of the total volume, and was ineffective in enmeshing red cells. By contrast, normal blood produced a large firm clot occupying the whole volume

and enmeshing almost all the red cells. When the original specimen sent for cross-matching was substituted for the oxalated specimen no clot at all was seen. Presumably such fibrinogen as had been present had already clotted.

Treatment of the bleeding

Dried fibrinogen fractionated from pooled human plasma was injected intravenously as fast as possible. Four grams were dissolved in 200 ml. of pyrogen-free distilled water and given over 20 minutes. At the time, the patient was dangerously shocked, with blood running freely from the wound, and he had a weak pulse of 130 a minute. All serious bleeding ceased within half an hour, and within one hour of the fibrinogen transfusion all oozing had stopped, with dramatic clinical improvement. No further fibrinogen therapy was necessary. The wound subsequently healed well.

Investigations for fibrinolysis

The simplest emergency method for demonstrating fibrinolysis is by the observation of a whole blood clot kept at body temperature. This is always impossible when, as here, the fibrinogen level is so low that virtually no clot is formed. Further, the absence of patient's fibrinogen demands a test for fibrinolysis which uses fibrinogen from another source. No such technique was used here during the night of the emergency. Blood removed 12 hours after the

emergency was examined as follows.

Quantitative test for fibrinolysis (Biggs and Macfarlane, 1953b): Serial estimation of the amount of clot remaining after incubation (measured by "tyrosine" released) showed no evidence of commencing fibrinolysis up to four hours. The blood was not kept chilled before the test. A similar test performed on a specimen removed from the patient 90 hours after the emergency was not significantly different.

Examination for lysis of normal clot by patient's plasma: No lysis was visible to the naked eye following the immersion of a washed fibrin clot (formed by the addition of 0.2 ml. thrombin to 0.1 ml. normal plasma) into undiluted patient's plasma, followed by incubation at 37°C for 24 hours.

Subsequent course

The patient was discharged from hospital three weeks after the surgical episode from which he so nearly died. His wound had healed and the plasma fibrinogen had remained well within the normal range. The total serum acid phosphatase level had fallen progressively to 7 Gutman units per 100 ml. The blood urea was within the normal range. Stilboestrol had been started ten days after the operation and he was discharged home to take 10 mg. three times daily. Details of laboratory findings can be found in table XI.

Second admission

Six months later he was re-admitted on 8.10.56.

suffering from acute retention of urine. Immediately after his arrival in hospital he managed to pass urine spontaneously. Subsequent cystoscopy showed many small stones and phosphatic debris in the bladder. This required three sessions with a Bigelow's bladder evacuator to remove all the stones. At none of these occasions was there any clinical or haematological deterioration. Plasma fibrinogen level remained normal. Stilboestrol was continued as before.

Third admission

Within a month he was re-admitted on 25.11.56. again suffering from urinary retention. The cause was apparently the impaction of calcarious matter in his urethra. A cystoscope was passed twice on this occasion and a considerable amount of debris was removed from the bladder. In addition to maintaining the urine at an acid pH, stilboestrol was continued and he was discharged home on 10.12.56. It is worth noting that about this time he complained of swelling of his right leg below the knee. It became "blue" shortly afterwards. No cause for this was found and the leg recovered. It gave no further trouble until the end.

For the subsequent eighteen months the patient continued to attend the out-patient department at intervals. There was general satisfaction all round at his progress. At the end of this time he gave a story which was interpreted as the passing per urethram of a calculus which had been causing

ureteric colic. X-ray showed two large bladder calculi for which hospital treatment was advised.

Fourth admission

On 25.8.58. he was re-admitted for removal of bladder calculi. In the absence of any obvious haematological contraindication, supra-pubic cystostomy was performed and two large stones were removed. There was no unexpected bleeding at any time and healing was uneventful. The fibrinogen level was normal, but the rate of fibrinolysis had increased (see table XI).

Fifth admission

On 12.2.59. he was admitted as an emergency case. He had suffered since his previous discharge from epigastric pain passing through to the back, and coming on two hours after food. The pain was relieved by alkalis. Two nights before admission he had begun to cough up bright red blood and believed that he must have lost two pints altogether since then. There had been two similar but less severe periods of haemoptysis during the preceding week. He also commented that he had begun to bruise at the slightest touch for the previous two or three months. X-ray of the chest showed a density in the left lower lobe, possibly consolidation, and also many bony metastases. On this admission hypo-fibrinogenaemia was demonstrated for the first time since the acute episode of 1956, but it was accompanied by a thrombocytopenia of 40,000/c.mm., this for the first time

and a fast rate of clot lysis. Four grams of fibrinogen, fractionated from human plasma, were given with some good effect. The haemoptysis diminished to a minimal blood streaking, but the fibrinogen level relapsed to pre-treatment level within two days. 40 units of long-acting corticotrophin were given by injection every day in the hope of reducing the fibrinolysis. The patient's general state improved slightly and he was discharged on 3.3.59. feeling better, but not fit.

On 21.3.59. he died at home. The patient had been feeling quite well and was standing up to dust his pillow when he turned back and fell dead. His family doctor said that the patient had had further swelling in the right calf with some pain. There was pitting oedema of both legs, particularly on the right side. He thought the local diagnosis was of deep phlebothrombosis.

No autopsy was possible. It was suspected that he had died of a massive pulmonary infarct secondary to the suspected phlebothrombosis.

APPENDIX TWO

TABLE XXXII

The lysis times and plasma fibrinogen levels according to age, in the three control groups

No. of patients	Decade of age	Lysis time P_{50} (hours)	Plasma fibrinogen g./100 ml.
		Benign hypertrophy of the prostate	
2	50 - 59	118	0.28
		71	0.64
11	60 - 69	53	0.56
		+ 96	0.56
		67	0.70
		118	0.38
		++	0.53
		+ 71	0.33
		95	0.53
		69	0.32
		83	0.60
		92	0.48
		+ 22	0.54
6	70 - 79	21	-
		43	0.49
		45	0.49
		31	0.47
		67	0.26
		94	0.47
2	80 - 89	23	0.65
		++	0.43
		Miscellaneous disorders (none with known malignancy)	
7	20 - 29	212	0.31
		79	0.39
		86	0.27
		137	0.49
		176	0.68
		128	0.27
		++	0.25
8	30 - 39	118	0.23
		224	0.27
		73	0.57
		70	0.52
		78	0.40
		165	0.27
		105	0.25
		++	0.56

(Continued)

TABLE XXXII (cont.)

No. of patients	Decade of age	Lysis time P_{50} (hours)	Plasma fibrinogen g./100 ml.
(Miscellaneous disorders-none with known malignancy: (cont.))			
8	40 - 49	95	0.81
		34	0.54
		+ 72	0.45
		75	0.52
		+ 71	0.62
		55	0.70
		66	0.46
		+ 54	0.52
13	50 - 59	++	-
		71	0.36
		65	0.35
		93	0.31
		95	0.34
		23	0.53
		86	0.27
		48	0.38
		+ 31	0.33
		59	0.52
		83	0.38
		71	0.31
		+ 47	0.38
22	60 - 69	+ 41	-
		16	0.43
		99	0.33
		80	0.38
		28	0.32
		36	0.46
		176	0.86
		85	0.39
		57	0.43
		+ 97	0.37
		+ 49	0.27
		52	-
		30	0.60
		80	-
		++	0.90
		+ 120	0.60
		35	0.58
		78	-
		22	0.52
		71	0.46
		+ ++	1.00
		76	0.75

(Continued)

TABLE XXXII (cont.)

No. of patients	Decade of age	Lysis time P_{50} (hours)	Plasma fibrinogen g./100 ml.
(Miscellaneous disorders - none with known malignancy: (cont.))			
2	70 - 79	66 69	0.29 0.50
2	80 - 89	47 39	0.37 0.17
Miscellaneous disorders (all with known malignant disease elsewhere than in the prostate)			
1	20 - 29	80	0.66
3	30 - 39	72 129 33	0.30 0.75 0.54
10	40 - 49	54 21 58 ++ 120 75 167 98 47 120	0.47 0.31 - 0.69 0.26 0.55 0.46 0.31 0.35 0.66
9	50 - 59	27 40 74 58 119 74 70 71 33	0.38 0.66 0.55 0.38 0.79 0.43 0.55 0.44 0.50
17	60 - 69	101 + 48 99 37 59 71 43 64	0.73 0.18 0.52 0.52 0.49 0.66 0.89 0.52

(Continued)

TABLE XXXII (cont.)

No. of patients	Decade of age	Lysis time P_{50} (hours)	Plasma fibrinogen g./100 ml.
(Miscellaneous disorders - all with known malignant disease elsewhere than in the prostate (cont.))			
	60 - 69 (cont.)	+ 47	-
		69	0.32
		82	0.44
		10	1.06
		48	0.48
		81	0.64
		31	0.47
		+ 96	0.88
		65	0.20
13	70 - 79	71	0.46
		59	0.45
		48	0.57
		90	-
		56	-
		104	-
		59	-
		61	0.48
		36	0.52
		46	0.38
		18	0.54
		47	0.81
		35	0.55
2	80 - 89	79	1.16
		22	0.28

+ = buffer containing calcium

TABLE XXXIII

The lysis times and plasma fibrinogen levels according to age in the main series of carcinoma of prostate.

No. of patients	Decade of age	Lysis time in hours P_{50}			Plasma fibrinogen g./100 ml.
		Histological evidence of prostatic carcinoma	No histological evidence but diagnosis beyond reasonable doubt	Diagnosis by clinical story and palpation of prostate	
8	50 - 59	+ # ++			0.36
		+ 45			0.45
		72			0.65
		94 #			0.36
		+ 98 #			0.48
		+ 23			0.45
			+ # 55 #		0.47
			+ 42 #		0.65
23	60 - 69	47			0.29
		53			0.53
		+ 72			-
		36			-
		+ ++			0.57
		# 8			0.46
		35			0.48
		+ 77			0.53
		70			0.38
		68 #			0.26
		52 #			0.90
		52 #			-
		+ 50			0.66
		+ 31			0.53
		72 #			0.64
		94 #			-
		120			0.28
		+ # 120			0.59
			46		-
			56 #		0.48
				69	0.30
				+ 77	0.23
				+ 41	0.22
30	70 - 79	+ 95			-
		+ # 14 #			0.43
		# ?			0.32
		+ 72			0.54
		# 34 #			0.40
		# 20			-
		69			0.61
		69			0.64
		7			0.26
		144			0.38
		+ 37			0.68
		59 #			0.42
		++			0.61
		31			0.57
		22			-
		++ #			0.72
		+ 69			0.53
			62 #		0.66
			# 24 #		0.60
			+ 33 #		0.74
			19 #		0.61
			++		0.21
			23		0.56
			# 21 #		0.42
			# 23 #		0.40
			?		0.98
			++		-
				86	0.94
				23	0.45
				28	0.48
7	80 - 89	+ 48			0.58
		4			0.49
		+ 34 #			0.30
			# 72		0.30
			63		0.65
			21 #		-
				+ # 24	0.44

before figure signifies out-patient examination

after figure signifies the presence of known metastases

+ = buffer containing calcium

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